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EFFECT OF PHOSPHORUS ON THE COMPOSITION OF THE TOMATO PLANT [1]

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INTRODUCTION

A large amount of research has been undertaken in the attempt to determine the effects of phosphorus on plant growth. Experiments with tomatoes have been confined almost wholly to field plots. There is obvious need of repeating some of this experimentation in sand cultures, where it is possible to control the environment more closely and partially escape the complexity of factors found under field-plot conditions. On account of the importance of chemical analyses of plant tissues in the study of the effect of different environments on plant metabolism, an attempt was made to apply this method of investigation in determining the effects of variations in the supply of phosphorus on the growth and fruitfulness of the tomato (*Lycopersicon esculentum* Mill.). This paper reports some of the results of such an attempt.

METHODS

Tomato seed, of the Bonny Best variety, was used in all experiments and was obtained as commercial package stock from a seed company in Madison, Wis. The seeds were sown in greenhouse soil in flats, and the seedlings were transplanted to soil in 3-inch pots several days after the appearance of the first true leaf. Two weeks before they were transplanted to sand, the plants were shifted to 4-inch pots. The plants used for experimental purposes were about 15 inches high, and flower buds were making their appearance.

When the plants were transplanted to sand in the first experiment, the soil was washed from the roots, and the secondary roots were removed with a pair of shears. These secondary roots were cut off because of the large amount of time it would require to free them from all soil and organic matter, and also because the decay of these roots would add organic matter to the sand. The plants send out adventitious roots from the stems for their subsequent development. In the later experiments, by properly hardening the plants it was possible to cut the stems off at the surface of the ground and by removing some of the lower leaves, to transplant them directly to

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the sand as cuttings (fig. 1). The sand used was furnished by a sand company in Ottawa, Ill. This sand contained 99.8 per cent silica. The plants were grown in 2-gallon glazed jars having a drainage hole at the bottom on one side. Two plants were grown in each pot to which phosphorus was added, and either three or four plants were grown in each pot to which phosphorus was not added. The sand was kept moist enough to encourage plant growth by applying city tap water as frequently as necessary.

The leaves were kept dry by watering the plants carefully and by using wire screens to keep the leaves above the sand (*9*).[3] All fallen leaves were saved. The following modification of Knop's nutrient solution was used.

FIG. 1.—Plants in an ideal condition for transplanting by cuttings. Secondary roots have developed on the stem above the ground

Solution 1—Plus-phosphorus solution:
(A) Magnesium sulphate, 2 per cent.
Potassium nitrate, 2 per cent.
Monobasic potassium phosphate, 2 per cent.
(B) Calcium nitrate, 4 per cent.
Calcium sulphate, 2 per cent.
Calcium chloride, 3 per cent.

Solution 2—No-phosphorus solution:
(A) Magnesium sulphate, 2 per cent.
Potassium nitrate, 2 per cent.
Potassium chloride, 2 per cent.
(B) Calcium nitrate, 4 per cent.
Calcium sulphate, 2 per cent.
Calcium chloride, 3 per cent.

The above stock nutrient solutions were diluted for use before application by mixing 1 part of A, 1 part of B, and 10 parts of tap water. Once or twice a week the jars were treated with this diluted solution, to which was added 4 c. c. per liter of a 1 per cent solution of ferric citrate. Some plants were given solution 2, which contained no phosphorus; while other plants were given solution 1, which contained an ample supply of phosphorus. In experiment H the low-phosphorus plants were given an insufficient supply of phosphorus by using a solution composed of 9 parts of solution 2 and 1 part of solution 1. All flowers were hand-pollinated.

[3] Reference is made by number (italic) to "Literature cited," p. 126.

PRESERVATION OF SAMPLES AND PREPARATION FOR ANALYSIS

The plants were cut for samples about 1 p. m. on a sunny day. The leaves and stems were preserved immediately, usually before 2 p. m. The remaining tissue was preserved as soon as possible. The samples, after being cut into half-inch pieces, were dried for 30 minutes at 98° C. in a steam oven, and still further dried at 60° to 65°, being well ventilated until thoroughly dried. The drying process usually required 24 hours. Samples of roots were obtained by overturning the pots, removing the roots, and washing them moderately with water. No attempt was made to wash out all the sand before drying them. After the roots were dry they were ground gently in a large mortar with a pestle until they were free from sand. The material was placed on a large sheet of paper and the roots and sand were separated by blowing the roots away from the sand.

The dried material was ground with an electric-driven pestle mill until the material was of sufficient fineness to pass through a sieve with 60 meshes to the linear inch.

One-gram samples were dried at 100° C. for 48 hours before being weighed, and after another 20 hours of drying they were reweighed. In all cases a vacuum was used during the last hour of the drying.

One gram of plant material was placed in a porcelain crucible, and 15 c. c. of saturated alcoholic solution of magnesium nitrate was added. This was then dried at 65° C. A large part of the carbon was expelled by igniting the dried material with a fuse of quantitative filter paper before the material was placed in an electric furnace in which it was heated until all the carbon had disappeared. The analysis was completed by the official volumetric method of the Association of Official Agricultural Chemists. The phosphomolybdate precipitate was filtered on a Gooch crucible, a paper-pulp mat being used.

One gram of dry powder was used to determine the total nitrogen by the official Kjeldahl-Gunning-Arnold method.

QUANTITATIVE SEPARATION OF NITROGENOUS CONSTITUENTS

Approximately 200 grams of fresh plant tissue were weighed out and chopped into fine pieces in a wooden bowl. Three 15-gram samples were weighed out in evaporating dishes for moisture determination, and three 5-gram samples were similarly weighed out for total nitrogen determination. One hundred grams of the tissue was crushed in a large mortar with a pestle. About 15 c. c. of ether was added to plasmolyze the cells, and angular quartz sand (previously washed and dried) was added to facilitate the grinding. The tissue was ground to a fine pulp, usually with the addition of more ether, and water if necessary.

The product of the above procedure was transferred to a piece of long cloth on a large funnel and was extracted by applying 2 liters of distilled water in about 30 washings. After the extraction, the solution was filtered through paper pulp and filter paper on a Büchner funnel, and made up to a definite volume of 2 liters.

The nitrogen constituents were determined on the following fractions by the total-nitrogen method previously mentioned: 150 c. c. of the above solution was used for the determination of water-soluble nitrogen; 300 c. c. aliquots were heated to boiling, 2 c. c. of 10 per

cent acetic acid was added, and the mixture was boiled for two minutes, then filtered hot into a fluted filter and washed with 100 c. c. of hot water, and the total nitrogen determined both on the coagulum and the filtrate; 600 c. c. of this filtrate was made up to a liter in volume and preserved by the addition of chloroform and toluene until the next day, when the nitrates were determined by the Strowd method (*14*).

Between 3 and 5 grams of dry material was placed in a 300 c. c. Erlenmeyer flask and was extracted with 90 per cent alcohol under a reflux condenser on an electric hot plate. The alcohol was kept boiling for two hours. This material was filtered and washed with an equal volume of boiling 90 per cent alcohol. The extract was evaporated on an electric hot plate at about 65° C., water being added until the extract was free from alcohol. The solution was cleared with neutral lead acetate, nearly deleaded with sodium sulfate, and the deleading was completed with sodium carbonate, phenolphthalein being used as an indicator. By the use of phenolphthalein a very distinct termination of the deleading can be easily seen. The solutions were at all times filtered through dry filter paper into dry vessels. The solution was made up to volume and the reducing power determined.

One-half of the solution prepared for reducing sugars was boiled for 45 minutes with 2½ per cent hydrochloric acid for total hydrolysis of sugars. The solution was neutralized, made up to volume, and the reducing power determined.

The dry material from which the sugar had been extracted, as indicated above, was placed in a beaker, and 50 c. c. of water was added. The dry material was well mixed with the water and then allowed to stand at room temperature for 12 hours or overnight. After the material had been filtered and washed with cold water, the filtrate was boiled for two and one-half hours on a sand bath with 2½ per cent hydrochloric acid. The solution was neutralized, clarified, and deleaded, and the reducing power was determined on the filtrate after it was made up to volume. It is assumed that this fraction contains the dextrins and soluble starch.

After the water had been extracted the residue was placed in the previously used beaker with 50 c. c. of water and brought to boiling and kept boiling for two minutes under a low flame to gelatinize the starch. The material was cooled to 38° C. by using a water bath, and 10 c. c. of fresh saliva (not over one hour old) was added. The hydrolysis of the starch was allowed to continue until testing with iodine solution showed that the starch had disappeared. The material was again brought to a boil in order to test for completeness of hydrolysis and to facilitate filtration. After it had been filtered, the solution was boiled for two and one-half hours on a sand bath with 2½ per cent hydrochloric acid. As clarification was usually not necessary, the solution was neutralized, made up to volume, and the reducing power determined.

The residue was washed into an Erlenmeyer flask with 2½ per cent hydrochloric acid and boiled for two and one-half hours on a sand bath. The solution was filtered, neutralized, clarified, and made up to volume, and the reducing power determined. This fraction is called hemicellulose in all tables.

The reducing power of all carbohydrate solutions was determined by obtaining the reduced copper under the Munson and Walker conditions. The amount of reduced copper was determined by the Shaffer and Hartmann titration method (*12*). The tables for the Munson and Walker conditions were used to find the copper equivalent in glucose. All carbohydrates are expressed in percentages of glucose.

Fresh material was cut into pieces one-half inch in length and placed in a fixative of 4 c. c. of 4 per cent formaldehyde and 96 c. c. of 60 per cent alcohol. The material was cut in paraffin on a sliding microtome and stained with safranine.

RESULTS

Experiments A, B. C, and E were preliminary and are not reported here.

EXPERIMENT D

The seed for this experiment was planted July 9, 1923; the seedlings were transplanted to 3-inch pots July 21, 1923; and to 4-inch pots August 3, 1923. The plants were placed in sand under the nutrient treatment September 3, 1923. The no-phosphorus plants, 101 in number, were sampled for analysis October 25, 1923, and the ample-phosphorus plants, 50 in number, October 26, 1923 (fig. 2). Thus the groups had been under nutrient treatment 52 and 53 days, respectively.

In experiment D the material was separated into the following fractions: The plants as they were set out were divided into thirds according to the number of leaves; and at the end of the experiment each plant was divided into five regions of leaves and five regions of stem. The basal three regions were identical with the regions on the plants as set out. Thus the terms "bottom leaves" and "bottom stem" refer to the leaves on the bottom third of the original plant and the portion of the stem to which these leaves were attached. The "next to bottom" and "middle" refer to the successive thirds of the original plant toward the growing point. The "top" fraction consists of the uppermost three leaves and the corresponding portion of the stem, at the end of the experiment. The "next-to-top" portion is the region between the "top" and the "middle" portions and in the ample-phosphorus plants comprises the largest region of the plant.

In all cases where fruit was separated into seed and pulp the following method was used: A fruit was cut in cross section, and the seed, together with the surrounding placental pulp, was removed with a scalpel. This portion was analyzed with the seed; the remaining portion is referred to as pulp. This seems perhaps an unsatisfactory method, but it is exceedingly difficult to separate all the pulp from the seeds in fruits ¾ inch to 1 inch in diameter because the seeds are soft and small. In the case of ripe fruits the placental tissue can be freed from the seeds fairly satisfactorily by pressing the pulp surrounding the seeds through a piece of cheesecloth. This was not done in any of the experiments reported here.

In both treatments the lower leaves, which were yellow in color, fell from the plants during the course of the experiment. The bottom

leaf or two died within two weeks after transplanting; after a short time the other lower leaves fell successively until the end of the experiment. Most of the defoliation, however, was on the no-phosphorus plants. Under both treatments it was usual for a leaf to show first a dead region farthest away from the stem. These dead areas increased in size progressively toward the stem. The ventral

FIG. 2.—Plants receiving no phosphorus (left) and ample phosphorus (right)

surface of the leaves of the no-phosphorus plants also had a reddish appearance, probably due to the development of anthocyanin pigments in the subepidermal cells. The ample-phosphorus plants did not show any of these characteristics. It will be seen from Tables 1 and 2 that the plants in the two treatments differed as to weight of plant, amount of linear growth, and the size and number of fruits.

TABLE 1.—*Growth of tomato plants under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D*

Plants treated with—	Average green weight per plant			Average growth per plant
	Plant as set out	Plant less fruit at end of experiment	Fruit	
	Gm.	*Gm.*	*Gm.*	*Cm.*
No phosphorus	23.78	38.38	13.84	23.08
Ample phosphorus	23.73	179.28	119.09	53.80

TABLE 2.—*Blossom and fruit setting of tomato plants under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D*

Plants treated with—	Average number of blossoms pollinated per cluster				Average number of fruits set per plant	Average number of blossoms set or partially developed per plant
	Cluster No.					
	1	2	3	4		
No phosphorus	1.90	0.99			0.93	2.67
Ample phosphorus	3.60	4.10	0.93	0.12	3.46	8.22

In May, 1925, the remaining data for experiment D were obtained, which included fresh analyses of the nitrogenous constituents and the saving of the seed from vine-ripened fruits for growth tests, the results of which are given in Table 3.

TABLE 3.—*Growth of plants from the original tomato seeds used in experiment D as compared with growth of plants from seeds produced under ample-phosphorus and no-phosphorus nutrient treatments*

Seeds	Average weight of tops per plant grown in garden soil 31 days		Average weight of plants grown in sand 30 days and watered with nutrient solution 2 (which contained no phosphorus)		Average weight of 1 seed	Germination
	Green	Dry	Green	Dry		
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Mgm.*	*Per cent*
Original	0.793	0.080	0.313	0.017	3.30	68.5
No phosphorus	.996	.108	.213	.003	1.68	97.0
Ample phosphorus	1.095	.113	.460	.023	3.54	98.5

At the time experiment D was concluded seed were not saved from the two phosphorus treatments or fresh tissue analysis made. As these data were obtained for experiment F, it was thought advisable in the spring of 1925 to repeat the treatments and obtain these additional data. Two sets of plants were given no-phosphorus and ample-phosphorus nutrient treatment. Seed were saved from ripe fruits of these two treatments and the remaining tissue was used to obtain the data given in Table 7.

A sample of the seed used to start these plants was saved as well as the seed obtained from ripe fruits of the two treatments. It was thought of value to compare the potentialities for growth in these three lots of seed. Germination tests were made under uniform conditions, and the average weight of a single seed was determined. The three lots of seed were sown in pure quartz sand which was watered with a solution containing no phosphorus to determine whether the reduced amount of phosphorus in the seed would have any effect on the amount of growth produced. The seeds were also planted in garden soil to determine whether all lots of seed could produce equally vigorous plants. The growth tests were made in September, 1925.

The experiment was brought to a close because it was apparent that some of the no-phosphorus plants were likely to die from starvation.

In one case the treatment had been carried to such an extent that the plant died. Three plants of the no-phosphorus series were subjected to a treatment of phosphorus supposedly sufficient to restore metabolism, but all died without resuming growth. The roots of the no-phosphorus plants were much shorter than those of the ample-phosphorus plants and were dark brown in color, especially at the ends, whereas the roots of the plants that had had ample phosphorus were white.

Anatomical studies of the stems grown under these different nutrition conditions seem to indicate that there was no significant anatomical difference. The results obtained in Experiment D are shown in Tables 4 to 9, inclusive.

TABLE 4.—*Percentage of phosphorus in the different regions of tomato plants grown under ample-phosphorus and under no-phosphorus nutrient treatments—Experiment D* [a]

Plant regions	Percentage of phosphorus, based on dry weight, in—			Percentage of phosphorus, based on green weight, in—		
	Control plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants
Leaves:						
Bottom	0.302		0.349	0.0313		0.0296
Next to bottom	.355	0.109	.376	.0434	0.0138	.0358
Middle	.585	.105	.507	.0887	.0156	.0422
Next to top	[b] .978	.131	.523	[b] .1308	.0154	.0449
Top		.162	.662		.0211	.0608
Stems:						
Bottom	.262	.064	.189	.0247	.0077	.0222
Next to bottom	.335	.047	.189	.0274	.0055	.0233
Middle	.598	.049	.300	.0416	.0052	.0311
Next to top		.050	.413		.0051	.0347
Top		.190	.786		.0187	.0529
Cluster stems		.188	.543		.0241	.0620
Fruit (whole)		.388	.825		.0186	.0786
Taproot	.312	.111	.200	.0346	.0105	.0206
Secondary root		.165	.572		.0142	.0654
Fallen leaves:						
Bottom		.108	.352		.1027	.3356
Next to bottom		.101	.323		.0952	.3043
Middle		.109			.1040	

[a] The no-phosphorus plants were grown 52 days and the ample-phosphorus plants 53 days.

[b] Growing point, which consisted of terminal bud and adjacent leaf which was never more than 4 millimeters in length.

TABLE 5.—*Amounts of dry matter and phosphorus in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D* [a]

Plant regions	Percentage of dry matter in—			Grams of dry weight in—			Milligrams of phosphorus per plant in—		
	Control plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants
Leaves:									
Bottom	10.36	.....	8.47	0.559	.....	0.146	1.687	.....	0.509
Next to bottom	12.24	12.66	9.53	.666	0.054	1.201	2.262	0.059	4.511
Middle	15.17	14.82	8.33	.258	.381	1.449	1.508	.400	7.344
Next to top	[b] 13.37	11.75	8.56	[b] .034	.342	4.790	[b] .335	.449	25.040
Top	.....	13.06	9.17	.....	.106	.521	.....	.171	3.449
Stem:									
Bottom	9.47	11.95	11.77	.392	.418	.685	1.027	.268	1.293
Next to bottom	8.18	11.68	12.32	.269	.408	.710	.901	.191	1.342
Middle	6.96	10.66	10.39	.038	.242	.752	.225	.119	2.253
Next to top	.....	10.16	8.39	.....	.172	1.981	.....	.086	8.186
Top	.....	9.82	6.72	.....	.128	.044	.....	.243	.349
Cluster stem	.....	12.87	11.43	.....	.098	.464	.....	.184	2.518
Fruit	.....	9.56	4.68	.....	1.323	9.808	.....	5.135	80.900
Taproot	11.10	9.50	10.27	.210	.572	.597	.769	.633	1.194
Secondary root	.....	8.63	11.43	.....	.325	3.890	.....	.534	22.240
Fallen leaves:									
Bottom	.....	85.04	89.36	.....	.655	.549	.....	.710	1.929
Next to bottom	.....	89.11	84.87	.....	.623	.052	.....	.630	.017
Middle	.....	84.91	.....	.....	.177	.....	.....	.193	.....
Phosphorus in plant	.....	.....	.....	.....	.....	.....	.....	10.00	163.07
Phosphorus in water	.....	.....	.....	.....	.....	.....	.....	.29	.29
Total weight	.....	.....	.....	2.43	6.02	27.64	8.714	9.71	162.78
Total phosphorus per plant (1 analysis)	.....	.....	.....	.....	.....	.....	8.74	.....	.....

[a] The no-phosphorus plants were grown 52 days and the ample-phosphorus plants 53 days.
[b] Growing point, which consisted of terminal bud and adjacent leaf which was never more than 4 millimeters in length.

TABLE 6.—*Percentage of nitrogen in the different regions of tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D* [a]

Plant regions	Percentage of nitrogen, based on dry weight, in—			Percentage of nitrogen, based on green weight, in—		
	Control plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants
Leaves:						
Bottom	2.68	.....	1.30	0.300	.....	0.111
Next to bottom	3.43	3.27	1.79	.447	0.413	.171
Middle	4.40	3.78	2.30	.714	.562	.192
Next to top	.....	4.07	2.96	.....	.477	.254
Top	.....	4.24	4.34	.....	.553	.397
Stem:						
Bottom	1.79	2.24	1.12	.179	.269	.132
Next to bottom	1.98	2.18	.92	.176	.255	.113
Middle	.....	2.23	.86	.....	.238	.089
Next to top	.....	2.36	1.35	.....	.240	.113
Top	.....	.....	3.60	.....	.....	.242
Cluster stem	.....	3.14	2.37	.....	.403	.271
Fruit	.....	3.84	3.50	.....	.184	.164
Taproot	1.82	2.42	1.37	.218	.231	.140
Secondary root	.....	2.50	2.80	.....	.216	.107
Fallen leaves:						
Bottom	.....	2.11	1.20	.....	1.820	1.040
Next to bottom	.....	2.70	1.23	.....	2.310	1.050

[a] The no-phosphorus plants were grown 52 days and the ample-phosphorus plants 53 days.

TABLE 7.—*Fresh tissue analysis for different nitrogenous constituents in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D*

PERCENTAGE OF NITROGEN BASED ON DRY WEIGHT

Plants	Total	Insoluble	Coagulum	Filtrate	Total soluble	Nitrates
As set out:						
Leaves	2.54	1.03	1.04	0.43	1.51	Trace.
Stems	1.48	.50	.23	.76	.98	0.37
No phosphorus:						
Leaves	3.12	1.89	.31	.83	1.23	.77
Stems	1.70	.92	.18	.64	.78	.46
Fruit	4.85	2.31	.49	2.02	2.54	.10
Ample phosphorus:						
Leaves	2.63	1.38	.83	.44	1.25	.10
Stems	.86	.41	.22	.22	.45	.09
Fruit	1.96	.37	.43	1.24	1.59	Trace.

PERCENTAGE OF NITROGEN BASED ON GREEN WEIGHT

As set out:						
Leaves	0.327	0.134	0.134	0.055	0.193	Trace.
Stems	.142	.047	.022	.073	.095	0.035
No phosphorus:						
Leaves	.619	.379	.060	.163	.240	.015
Stems	.274	.148	.030	.102	.126	.007
Fruit	.308	.150	.031	.128	.158	.001
Ample phosphorus:						
Leaves	.283	.148	.089	.047	.135	.011
Stems	.130	.062	.033	.033	.068	.013
Fruit	.152	.060	.025	.072	.092	Trace.

TABLE 8.—*Percentage of carbohydrates in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D*

[Based on dry weight]

AMPLE PHOSPHORUS IN THE NUTRIENT SOLUTION

Plant regions	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicellulose
Stem:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Bottom	1.68	4.57	0.80	0.74	12.99
Next to bottom	3.25	6.66	.94	1.16	12.87
Middle	4.50	9.33	1.04	1.17	10.11
Next to top	4.06	7.72	1.39	1.78	9.35
Leaves:					
Bottom	.88	2.62	.91	.81	5.00
Next to bottom	.61	1.38	1.22	.77	5.18
Middle	.69	1.40	.97	.56	5.70
Next to top	1.03	1.79	1.11	1.19	5.30
Top	1.33	2.28	1.13	2.06	5.77
Fallen leaves:					
Bottom	.47	.75	.74	.61	5.02
Next to bottom					
Middle					

NO PHOSPHORUS IN THE NUTRIENT SOLUTION

Stem:					
Bottom	2.68	3.72	0.86	0.67	10.35
Next to bottom	5.66	9.32	1.08	.62	11.73
Middle	7.31	10.00	1.45	.72	10.57
Next to top	6.44	9.37	1.16	.37	10.47
Leaves:					
Bottom					
Next to bottom	1.05	1.95	1.15	.21	6.66
Middle	1.14	1.83	1.56	1.02	6.27
Next to top	1.05	2.06	1.36	1.21	6.48
Top					
Fallen leaves:					
Bottom	.89	1.49	.75	.48	5.26
Next to bottom	1.01	1.75	1.02	.63	6.35
Middle	1.00	2.00	1.08	.82	5.93

TABLE 9.—*Percentage of carbohydrates in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D*

[Based on green weight]

AMPLE PHOSPHORUS IN THE NUTRIENT SOLUTION

Plant regions	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicellulose
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Stem:					
Bottom	0.196	0.535	0.094	0.087	1.522
Next to bottom	.400	.819	.116	.142	1.587
Middle	.470	.969	.108	.122	1.047
Next to top	.341	.647	.117	.149	.784
Leaves:					
Bottom	.131	.392	.136	.121	.746
Next to bottom	.058	.131	.117	.074	.494
Middle	.058	.117	.081	.049	.475
Next to top	.089	.154	.096	.102	.455
Top	.122	.211	.104	.190	.530
Fallen leaves:					
Bottom	.427	.670	.670	.553	4.510
Next to bottom					
Middle					

NO PHOSPHORUS IN THE NUTRIENT SOLUTION

Stem:					
Bottom	0.321	0.446	0.102	0.080	1.238
Next to bottom	.662	1.091	.126	.073	1.370
Middle	.778	1.062	.155	.076	1.127
Next to top	.654	.951	.117	.037	1.066
Leaves:					
Bottom					
Next to bottom	.267	.496	.292	.054	1.692
Middle	.169	.271	.231	.152	.931
Next to top	.123	.236	.161	.143	.761
Top					
Fallen leaves:					
Bottom	.746	1.265	.634	.410	4.75
Next to bottom	.900	1.550	.900	.559	5.63
Middle	.850	1.698	.912	.695	5.03

EXPERIMENT F

The seed for experiment F was planted February 2, 1924; the seedlings were transplanted to 3-inch pots February 15, 1924, and to 4-inch pots March 23, 1924; and the plants were placed in sand under nutrient treatment on April 11, 1924. The no-phosphorus plants, 89 in number, were sampled June 13, 1924, for preservation, and the ample-phosphorus plants, 40 in number, June 15, 1924. The plants had been under treatment for 63 and 65 days, respectively. A similar number of plants were sampled after 23 and 24 days of treatment in order to determine the rapidity of change in chemical composition. The same divisions of the plant were made in this experiment as in experiment D.

The general outward characteristics during phosphorus starvation were similar to those described under experiment D. Thus the no-phosphorus plants again differed from the ample-phosphorus plants as to chlorophyll appearance, development of anthocyanin pigments, weight of plant, amount of linear growth, and the size and number of fruits. (See Tables 10 and 11, and fig. 3.)

TABLE 10.—*Growth of tomato plants under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F*

Plants treated with—	Average green weight per plant			Average growth per plant
	Plant as set out	Plant less fruit at end of experiment	Fruit	
	Gm.	*Gm.*	*Gm.*	*Cm.*
No phosphorus	35.13	47.60	23.00	21.28
Ample phosphorus	37.43	186.13	206.91	73.30

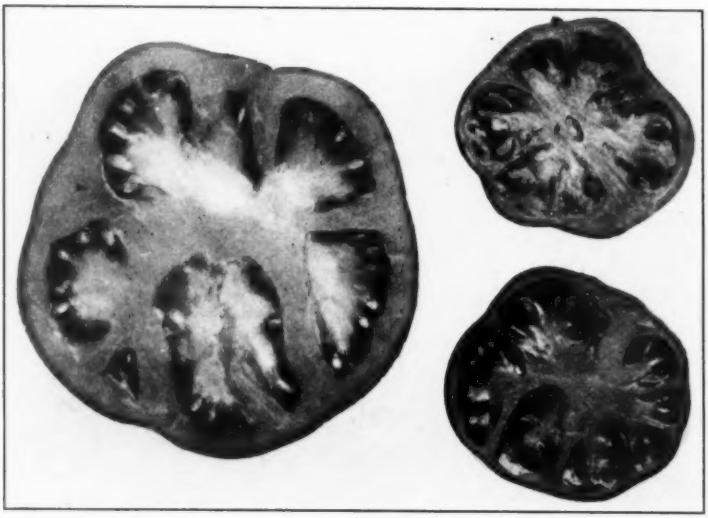

FIG. 3.—Fruits of plants receiving ample phosphorus (left) and no phosphorus (right) in experiment F

TABLE 11.—*Blossom and fruit setting of tomato plants under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F*

Plants treated with—	Average number of blossoms pollinated per cluster				Average number of fruit set per plant
	Cluster No.				
	1	2	3	4	
No phosphorus	1.55	0.83	0.00	.....	0.87
Ample phosphorus	2.85	1.65	.15	.33	3.70

The experiment was considered complete when two of the no-phosphorus plants had died. The roots were similar in appearance to those already described in experiment D. Six plants of the no-phosphorus series were used in determining what the response

would be to phosphorus applications after phosphorus starvation. The response was quite slow in becoming apparent, but was plainly visible after three to four weeks of treatment. These plants produced new growth, new flowers and fruits, and also ripened some fruits (fig. 4).

The anatomical studies of these stems show a marked increase in the amount of xylem in the ample-phosphorus plants as compared

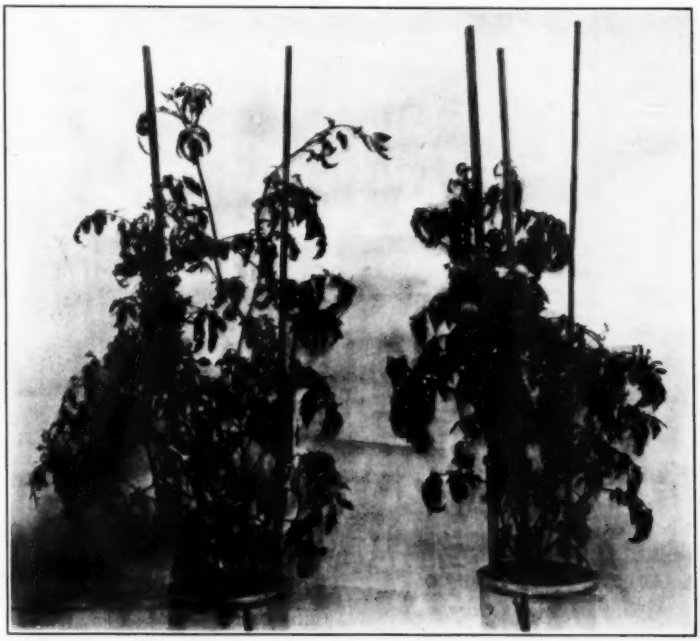

FIG. 4.—No-phosphorus plants after they had been treated for two months with the ample-phosphorus nutrient solution (experiment F)

with the no-phosphorus plants. In both experiments D and F the fascicular xylem was much more abundant than the interfascicular xylem in all sections.

Seeds from this experiment were saved from vine-ripened fruit in the usual way for separation of pulp from seed by fermentation. The growth tests were made in February, 1925, and the data given in Table 12 were obtained.

Other results obtained in experiment F are summarized in Tables 13 to 18.

TABLE 12.—*Growth of plants from original tomato seeds used in experiment F as compared with growth of plants from seeds produced under ample-phosphorus and no-phosphorus nutrient treatments*

Seeds	Average weight of tops per plant grown in garden soil 34 days		Average weight of plants grown in sand 37 days and watered with nutrient solution 2 (which contained no phosphorus)		Average weight of 1 seed	Germination
	Green	Dry	Green	Dry		
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Mgm.*	*Per cent*
Original	1.98	0.17	0.229	0.036	3.38	69.0
No phosphorus	1.90	.17	.098	.013	2.27	97.0
Ample phosphorus	2.72	.25	.271	.044	4.00	96.5

TABLE 13.—*Percentage of phosphorus in the different regions of tomato plants grown under ample-phosphorus and under no-phosphorus nutrient treatments—Experiment F* [a]

Plant regions	Percentage of phosphorus, based on dry weight, in—					Percentage of phosphorus, based on green weight, in—				
	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants
Leaves:										
Bottom	0.217	0.090	0.164		0.112	0.0172	0.0094	0.0177		0.0113
Next to bottom	.331	.138	.341	0.084	.295	.0301	.0145	.0368	0.0162	.0302
Middle	.543	.210	.514	.100	.313	.0620	.0273	.0605	.0172	.0315
Next to top				.104	.304				.0154	.0342
Top				.168	.483				.0257	.0556
Stem:										
Bottom	.204	.084	.236	.052	.162	.0175	.0102	.0261	.0072	.0208
Next to bottom	.390	.097	.267	.052	.145	.0110	.0103	.0266	.0067	.0187
Middle	.627	.122	.411	.056	.136	.0184	.0115	.0306	.0070	.0176
Next to top				.065	.193				.0074	.0204
Top				.127	.410				.0064	.0133
Cluster stem		.360	.738	.159	.282		.0522	.0506	.0242	.0402
Fruit pulp		[b].303	[b].538	.197	.488		[b].0278	[b].0446	.0108	°0289
Fruit seed				.346	.622				.0302	.0508
Taproot		.116	.237	.079	.237		.0127	.0247	.0090	.0236
Secondary root		.186	.376	.093	.399		.0104	.0173	.0064	.0256
Fallen leaves:										
Bottom		.103	.157	.091	.146		.088	.131	.0757	.125
Next to bottom		.126	.187	.082	.142		.104	.074	.0682	.116
Middle				.092	.142				.0770	.123

[a] The no-phosphorus plants supplying the data for the second and seventh columns of figures were grown 23 days; for the fourth and ninth columns of figures, 63 days. The ample-phosphorus plants supplying data for the third and eighth columns of figures were grown 24 days; for the fifth and tenth columns of figures, 65 days.

[b] Sample not divided into seed and pulp, but whole fruit analyzed.

TABLE 14.—*Amounts of dry matter and phosphorus in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F* [a]

Plant regions	Percentage of dry matter in—					Grams of dry weight in—					Milligrams of phosporous per plant in—				
	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants
Leaves:															
Bottom	7.86	10.33	10.65	.....	9.95	0.556	0.325	0.322	.....	0.265	1.21	0.296	0.527	.....	0.297
Next to bottom	9.07	10.49	10.72	19.20	10.27	1.005	.761	.953	.179	1.302	3.32	1.050	3.250	0.151	3.842
Middle	11.43	12.83	11.72	17.08	10.00	.619	.955	2.451	1.066	1.906	3.37	2.005	12.600	1.063	5.966
Next to top	.....	.....	.....	14.60	11.15	.....	.....	.....	.788	8.034	.....	.....	.....	.819	24.42
Top	.....	.....	.....	15.21	11.50	.....	.....	.....	.418	.927	.....	.....	.....	.702	4.479
Stem:															
Bottom	8.56	11.96	10.95	13.91	12.71	.528	.574	.407	.602	.610	1.08	.482	.961	.313	.988
Next to bottom	5.63	10.60	9.99	12.92	12.61	.297	.562	.391	.708	.737	1.16	.546	1.043	.365	1.068
Middle	5.81	9.41	7.42	12.32	12.82	.078	.455	.513	.398	.715	.49	.555	2.109	.224	.972
Next to top	.....	.....	.....	11.37	10.55	.....	.....	.....	.238	2.300	.....	.....	.....	.153	4.440
Top	.....	.....	.....	9.98	6.51	.....	.....	.....	.060	.140	.....	.....	.....	.076	.575
Cluster stem	.....	14.53	13.72	15.16	14.23	.....	.099	.083	.126	.417	.....	.357	.613	.200	1.176
Fruit pulp	.....	[b] 9.18	[b] 8.23	8.73	5.92	.....	.549	.343	1.459	8.915	.....	1.662	1.848	2.875	43.500
Fruit seed	.....	.....	.....	5.49	8.16	.....	.....	.....	.344	3.888	.....	.....	.....	1.191	24.180
Taproot	.....	10.84	10.45	11.32	9.95	.....	.331	.430	.713	.799	.....	.384	1.029	.550	1.894
Secondary roots	.....	5.55	4.58	6.87	6.37	.....	.248	.441	.648	1.301	.....	.461	1.656	.602	5.191
Fallen leaves:															
Bottom	.....	85.17	82.23	82.66	85.44	.....	.319	.261	.601	.475	.....	.329	.411	.547	.694
Next to bottom	.....	82.68	78.29	83.44	81.68	.....	.034	.067	.682	1.061	.....	.043	.013	.556	1.508
Middle	.....	.....	.....	84.12	86.80	.....	.....	.....	.241	.226	.....	.....	.....	.221	.321
Phosphorus in plant	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	8.17	26.17	10.61	125.51
Phosphorus in water	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.13	.13	.34	.34
Total weight	.....	.....	.....	.....	.....	3.083	5.212	6.663	9.270	34.018	10.63	8.04	26.04	10.27	125.17
Total phosphorus per plant (one analysis)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	9.8	.....	.....	.....	.....

[a] The no-phosphorus plants supplying data for the second, seventh, and twelfth columns of figures were grown 23 days; for the fourth, ninth, and fourteenth columns of figures, 63 days. The ample-phosphorus plants supplying data for the third, eighth, and thirteenth columns of figures were grown 29 days; for the fifth, tenth, and fifteenth columns of figures, 65 days.

[b] The sample was not divided into seed and pulp, but the whole fruit was analyzed.

TABLE 15.—*Percentage of nitrogen in the different regions of tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F* [a]

Plant regions	Percentage of nitrogen, based on dry weight in—					Percentage of nitrogen, based on green weight in—				
	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants
Leaves:										
Bottom	2.45	2.02	2.66		1.72	0.194	0.203	0.284		0.171
Next to bottom	3.37	2.66	3.16	2.90	2.04	.305	.276	.340	0.559	.209
Middle	4.55	3.64	4.36	3.54	2.46	.519	.466	.514	.608	.248
Next to top				3.94	3.10				.578	.348
Top				3.79	4.56				.578	.524
Stem:										
Bottom	3.92	1.66	1.82	2.19	1.57	.337	.199	.200	.305	.201
Next to bottom	2.00	1.81	2.11	2.21	1.45	.113	.192	.211	.286	.184
Middle	2.85	1.98	2.89	2.20	1.42	.165	.187	.214	.276	.183
Next to top				2.18	1.70				.247	.181
Top				2.72	3.00				.270	.196
Cluster stem		3.33	4.17	3.05	2.63		.483	.572	.462	.376
Fruit pulp		3.13	3.31	3.05	3.31		.285	.274	.168	.189
Fruit seed				3.76	3.87				.328	.315
Taproot		1.68	1.81	1.87	1.66		.182	.189	.212	.165
Secondary root		2.00	2.56	1.75	2.07		.111	.117	.120	.132
Fallen leaves:										
Bottom		1.36	1.56	1.53	1.57		1.160	1.200	1.270	1.340
Next to bottom		1.56	1.65	2.20	2.10		1.280	1.300	1.830	1.710

[a] The no-phosphorus plants supplying data for the second and seventh columns of figures average were grown 23 days; for the fourth and ninth columns of figures, 63 days. The ample-phosphorus plants supplying data for the third and eighth columns of figures were grown 24 days; for the fifth and tenth columns of figures, 65 days.

TABLE 16.—*Fresh-tissue analysis for different nitrogenous constituents in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F*

PERCENTAGE OF NITROGEN BASED ON DRY WEIGHT

Plants	Total	Insoluble	Coagulum	Filtrate	Water soluble	Nitrates
As set out:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Leaves	3.79	2.28	0.91	0.52	1.51	0.45
Stems	2.04	.84	.23	.79	1.20	.97
Treated 24 days with:						
No phosphorus—						
Leaves	3.37	2.67	.90	.58	.70	.66
Stems	1.77	.92	.43	.20	.85	.35
Ample phosphorus—						
Leaves	4.55	3.23	1.15	.71	1.32	.68
Stems	1.84	.81	1.03	.45	1.03	.83
Treated 64 days with:						
No phosphorus—						
Leaves	2.97	1.72	.18	1.21	1.25	.43
Stems	2.07	.69	.06	1.39	1.38	.32
Ample phosphorus—						
Leaves	2.97	1.74	.69	.58	1.23	.31
Stems	2.14	1.37	.80	.61	.77	.84

PERCENTAGE OF NITROGEN BASED ON GREEN WEIGHT

As set out:						
Leaves	0.363	0.219	0.088	0.050	0.144	0.044
Stems	.153	.063	.018	.059	.090	.073
Treated 24 days with:						
No phosphorus—						
Leaves	.441	.349	.118	.077	.092	.087
Stems	.251	.131	.061	.028	.120	.049
Ample phosphorus—						
Leaves	.520	.370	.131	.081	.150	.078
Stems	.245	.107	.137	.060	.138	.110
Treated 64 days with:						
No phosphorus—						
Leaves	.536	.317	.032	.212	.219	.075
Stems	.281	.094	.008	.189	.187	.043
Ample phosphorus—						
Leaves	.327	.191	.077	.064	.136	.034
Stems	.214	.122	.097	.073	.092	.101

TABLE 17.—*Percentage of carbohydrates in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F*

[Based on dry weight]

AMPLE PHOSPHORUS IN THE NUTRIENT SOLUTION

Plant region	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicellulose
Stem:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Bottom	2.85	6.53	1.85	2.36	12.35
Next to bottom	3.21	7.38	2.52	2.99	11.18
Middle	4.61	9.80	3.83	3.54	10.35
Next to top	4.20	10.58	1.71	4.36	9.77
Leaves:					
Bottom [a]					
Next to bottom	1.03	1.69	.79	1.73	5.57
Middle	1.28	2.11	.88	2.56	6.08
Next to top	1.48	2.55	.86	6.07	6.86
Top	1.14	2.67	1.15	4.76	7.45
Fallen leaves:					
Bottom	.75	1.55	1.09	.79	4.95
Next to bottom	1.23	1.86	1.45	.95	5.03

NO PHOSPHORUS IN THE NUTRIENT SOLUTION

Stem:					
Bottom	4.76	13.02	1.04	2.59	8.21
Next to bottom	6.39	16.93	1.44	1.64	9.66
Middle	8.58	18.90	1.16	1.82	9.55
Next to top	6.91	12.99	1.05	2.23	10.73
Leaves:					
Bottom [a]					
Next to bottom	2.90	4.88	.70	.92	5.52
Middle	1.95	2.40	1.07	.65	5.36
Next to top	1.52	2.05	1.16	1.09	6.22
Top	1.39	1.85	.93	3.28	7.41
Fallen leaves:					
Bottom	.62	1.02	1.09	.88	5.37
Next to bottom	1.03	1.04	1.34	.75	5.39

[a] There was an insufficient amount of bottom leaves for carbohydrate analysis.

TABLE 18.—*Percentage of carbohydrates in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F*

[Based on green weight]

AMPLE PHOSPHORUS IN THE NUTRIENT SOLUTION

Plant region	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicellulose
Stem:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Bottom	0.366	0.833	0.237	0.272	1.582
Next to bottom	.409	.939	.322	.380	1.420
Middle	.595	1.262	.494	.457	1.336
Next top	.446	1.122	.182	.463	1.038
Leaves:					
Bottom [a]					
Next to bottom	.104	.172	.081	.176	.566
Middle	.131	.215	.090	.256	.621
Next to top	.166	.287	.097	.680	.765
Top	.132	.308	.132	.553	.858
Fallen leaves:					
Bottom	.647	1.330	.981	.675	4.225
Next to bottom	1.014	1.509	1.184	.771	4.195

NO PHOSPHORUS IN THE NUTRIENT SOLUTION

Stem:					
Bottom	0.661	1.805	0.144	0.361	1.136
Next to bottom	.825	2.184	.184	.216	1.247
Middle	1.071	2.360	.145	.227	1.194
Next to top	.784	1.441	.119	.252	1.211
Leaves:					
Bottom [a]					
Next to bottom	.559	.938	.135	.177	1.060
Middle	.336	.411	.184	.112	.926
Next to top	.223	.302	.170	.160	.917
Top	.212	.281	.140	.501	1.125
Fallen leaves:					
Bottom	.515	.840	.907	.726	4.44
Next to bottom	.863	.864	1.120	.624	4.50

[a] There was an insufficient amount of bottom leaves for carbohydrate analysis.

EXPERIMENT H

The seed for experiment H was planted July 13, 1924; the plants were placed in sand under nutrient treatment September 11, 1924; the low-phosphorus plants, 26 in number, were sampled for preservation November 28, 1924, and the ample-phosphorus plants, 18 in number, November 29, 1924. The plants had been under treatment 78 and 79 days, respectively. The low-phosphorus plants received approximately 10 per cent of the amount of phosphorus given to the ample-phosphorus plants.

The general characteristics of the ample-phosphorus plants were similar to those of the plants used in experiments D and F, except that they were more mature and ripened more fruits than did the ample-phosphorus plants in experiments D and F. The low-phosphorus plants were smaller than the ample-phosphorus plants and set fewer fruits, and the ventral side of their leaves showed the characteristic anthocyanin pigments. Some of these low-phosphorus plants produced only one fruit, but that one was larger than the average fruits in either the low-phosphorus or the ample-phosphorus group. A few of the low-phosphorus plants did not show as uniform phosphorus limitation as the other low-phosphorus plants. Only one region of these plants which did not show uniform phosphorus limitation was taken for analysis—the next to top—which is so designated in the tables. Samples were taken of all the regions of the other low-phosphorus and ample-phosphorus plants, but the regions designated "bottom," "next to bottom," and "middle" were included in one sample.

Tables 19 to 23, inclusive, give the data obtained in this experiment.

TABLE 19.—*Growth of tomato plants under ample-phosphorus and low-phosphorus nutrient treatments—Experiment H*

Plants treated with—	Average green weight per plant			Average growth per plant
	Plant as set out	Plant less fruit at end of experiment	Fruit	
	Gm.	*Gm.*	*Gm.*	*Cm.*
Low phosphorus	13.91	128.33	167.06	76.77
Ample phosphorus	13.16	222.27	451.52	132.30

TABLE 20.—*Blossom and fruit setting of tomato plants under ample-phosphorus and low-phosphorus nutrient treatments—Experiment H*

Plants treated with—	Average number of blossoms pollinated per cluster								Average number of fruits set per plant	Average number of blossoms set or partially developed per plant.
	Cluster No.									
	1	2	3	4	5	6	7	8		
Low phosphorus	1.36	2.10	2.03	0.5	0.6	.....	.....	.....	2.26	6.0
Ample phosphorus	2.14	3.68	3.03	1.68	.5	1.47	.17	.1	6.38	10.5

TABLE 21.—*Percentage of nitrogen and phosphorus in the different regions of tomato plants grown under ample-phosphorus and low-phosphorus nutrient treatments—Experiment H*

[Based on dry weight and green weight]

Plant regions	Nitrogen				Phosphorus			
	Dry weight		Green weight		Dry weight		Green weight	
	Low phosphorus	Ample phosphorus	Low phosphorus	Ample phosphorus	Low phosphorus	Ample phosphorus	Low phosphorus	Ample phosphorus
Leaves:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Bottom-middle	2.06	1.42	0.253	0.157	0.153	0.358	0.0191	0.0395
Next to top	2.96	2.45	.322	.228	.155	.500	.0168	.0468
Next to top[a]	3.22		.299		.199		.0186	
Top	3.55	3.86	.425	.355	.207	.598	.0249	.0552
Fallen leaves:								
Bottom-middle	2.03	.94	1.840	.830	.079	.220	.0710	.1920
Stem:								
Bottom-middle	1.70	.96	.260	.123	.078	.177	.0120	.0228
Next to top	1.51	1.00	.168	.096	.067	.242	.0074	.0222
Next to top[a]	1.86		.164		.162		.0143	
Top					.157	.482	.0042	.0151
Cluster stem	2.68	2.03	.217	.280	.199	.476	.0322	.0659
Roots	1.95	1.50	.113	.093	.104	.381	.0060	.0236
Fruit:								
Green seed	3.66	3.83	.249	.211	.316	.645	.0215	.0357
Green pulp	2.86	2.95	.119	.118	.196	.550	.0082	.0215
Ripe seed	3.80	3.62	.285	.274	.615	.646	.0461	.0491
Ripe pulp	3.04	3.23	.109	.143	.394	.619	.0141	.0273

[a] Plants which did not show effects of phosphorus starvation as indicated by the lack of red pigment on the ventral side of leaves.

TABLE 22.—*Fresh-tissue analysis for nitrogen constituents in tomato plants grown under ample-phosphorus and low-phosphorus nutrient treatments—Experiment H*

PERCENTAGE OF NITROGEN BASED ON DRY WEIGHT

Plants	Total	Water insoluble	Coagulum	Filtrate	Water soluble	Nitrates
As set out:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Leaves	2.94	1.84	0.62	0.55	1.10	0.14
Stems	1.84	.62	.22	.98	1.22	.37
Low phosphorus:						
Leaves	2.85	1.40	.67	.82	1.45	.56
Stems	1.91	.91	.22	.85	1.01	.47
Fruit	3.07	1.27	.15	1.68	1.80	
Ample phosphorus:						
Leaves	2.31	1.45	.43	.53	.87	.13
Stems	.95	.50	.20	.30	.45	.11
Fruit	2.79	1.67	.05	1.04	1.12	

PERCENTAGE OF NITROGEN BASED ON GREEN WEIGHT

As set out:						
Leaves	0.452	0.288	0.092	0.082	0.164	0.021
Stems	.225	.075	.027	.120	.150	.045
Low phosphorus:						
Leaves	.334	.164	.079	.096	.170	.065
Stems	.222	.105	.025	.099	.117	.055
Fruit	.197	.080	.009	.109	.117	
Ample phosphorus:						
Leaves	.242	.151	.045	.055	.091	.013
Stems	.121	.064	.025	.039	.057	.012
Fruit	.176	.169	.003	.007	.007	

TABLE 23.—*Percentage of carbohydrates in tomato plants grown under ample-phosphorus and low-phosphorus nutrient treatments—Experiment H*

PERCENTAGE OF CARBOHYDRATES BASED ON DRY WEIGHT

Plant regions	Carbohydrates in low-phosphorus plants					Carbohydrates in ample-phosphorus plants				
	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicellulose	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicellulose
Leaves:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Bottom										
Middle	1.59	2.49	1.18	1.84	5.38	1.00	2.06	0.89	0.89	4.95
Next to top	2.29	3.29	.89	5.50	7.09	1.16	2.04	.76	1.56	7.20
Do.[a]	1.36	3.12	1.43	4.66	5.87					
Fallen leaves:										
Bottom										
Middle	.98	2.87	1.22	1.08	5.29	.79	2.17	1.02	1.04	4.93
Stem:										
Bottom										
Middle	4.48	9.07	1.73	1.88	11.49	2.84	5.74	.84	.94	12.13
Next to top	8.12	12.50	1.18	1.25	11.10	3.80	6.38	1.08	1.05	10.12
Do.[a]	4.19	7.87	.94	1.47	8.86					

PERCENTAGE OF CARBOHYDRATES BASED ON GREEN WEIGHT

Leaves:										
Bottom										
Middle	0.199	0.315	0.147	0.231	0.672	0.109	0.227	0.097	0.097	0.539
Next to top	.250	.361	.096	.596	.769	.108	.193	.071	.146	.671
Do.[a]										
Fallen leaves:										
Bottom										
Middle	.886	2.610	1.113	.982	4.760	.686	1.887	.908	.908	4.280
Stem:										
Bottom										
Middle	.685	1.383	.264	.288	1.752	.364	.734	.109	.120	1.556
Next to top	.902	1.383	.130	.139	1.232	.364	.607	.103	.101	.972
Do.[a]										

[a] Plants which did not show effects of phosphorus starvation as indicated by red pigment on under side of leaves.

RIPENING EXPERIMENT

It seemed advisable to make some analyses of tomatoes at different stages of growth to determine the distribution of phosphorus in the fruit. Plants for this experiment were set in the greenhouse bench during the spring of 1924 and in a different bench in the summer of 1924. To insure a difference in soil fertility in the two tests, the soil used in the second test was supplied with a liberal quantity of sheep manure and steamed bone meal. These two experiments are referred to according to the time the fruit ripened as April–June and August–September (Table 24).

There is difficulty in determining the stages of ripening in the tomato; the following method, however, was used successfully. The first stage was based on the date of pollination and the size of the fruit—3 to 5 mm. in diameter three to five days from pollination. It was necessary to take the date into consideration so that no fruits would be included which had started ovary development, but would never produce a mature fruit. The second stage was based wholly on the size of the fruit, that is, ¾ inch to 1 inch in diameter. The fruit of this stage was approximately three weeks old. The number of days from pollination to the ripening of the fruit fluctuates so much as to make such a basis of questionable value in the more mature

stages. The other three stages, therefore, were determined according to the consistency of the fruit as shown by testing in the palm of the hand for hardness and according to the color of the fruit. The more advanced stages were hard green, soft green, and ripe. The method used in separating fruits into pulp and seed and surrounding pulp is described in experiment D.

TABLE 24.—*Phosphorus content of tomatoes at different stages of growth—plants grown in garden soil*

Material and stages	Percentage of phosphorus based on—				Total amount of phosphorus per fruit		Total weight per fruit			
	Dry weight		Green weight				Dry weight		Green weight	
	April–June	August–September	April–June	August–September	April–June	August–September	April–June	August–September	April–June	August–September
Pulp:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Mg.*	*Mg.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
First stage (3 to 5 mm. in diameter)[a]										
Second stage (¾ to 1 inch in diameter)	0.571	0.561	0.0410	0.0490	1.55	1.27	0.272	0.227	3.79	2.65
Third stage (hard green)	.465	.589	.0378	.0280	11.38	10.81	2.452	1.835	81.08	38.51
Fourth stage (soft green)	.361	.646	.0157	.0219	21.85	20.17	6.072	3.123	138.97	91.73
Fifth stage (ripe)	.362	.576	.0174	.0288	26.18	24.86	7.232	4.316	150.24	86.14
Seed and surrounding pulp:										
First stage (3 to 5 mm. in diameter)	1.570	1.400	.0017	.0015	.053	.054	.0035	.0038	.0304	.0348
Second stage (¾ to 1 inch in diameter)	.793	.805	.0244	.0261	1.205	1.123	.152	.1394	2.44	2.15
Third stage (hard green)	.683	.666	.0380	.0348	7.957	5.350	1.165	.803	20.98	15.44
Fourth stage (soft green)	.582	.633	.0398	.0405	17.17	10.98	2.951	1.735	43.25	27.05
Fifth stage (ripe)	.567	.777	.0372	.0309	16.31	15.01	2.876	1.932	43.78	48.61

[a] Pulp and seed were not separated in this sample.

The changes in cellular structure during the ripening process are shown by the accompanying drawings (figs. 5 and 6). A fruit may be divided into the carpel, the placental region, and the developing seed. The carpel and the placental region undergo changes in size of cells and also a progressive disintegration of the cell walls. At the time of pollination the ovules are not surrounded by placental tissue; but there is a very active division and enlargement so that in a very short time, probably not more than a week after pollination, the ovules are surrounded by the placenta. The cells composing the placental outgrowth begin disintegration by becoming gelatinized. This process begins, in some cases at least, within three weeks after pollination. Disintegration occurs next in the more compact outer portions of the placenta below the level of attachment of the seeds. Finally some of the cells of the carpel walls disintegrate also. In both of these regions the cells enlarge enormously before disintegrating. Little or no change takes place in the vascular region, as these cells can be found intact in a fully ripened

fruit. Comparisons between the fruits of different ages, however, should be made primarily on the basis of size of cells, because different fruits as well as the same fruit, may differ as to the thickness of the carpel wall.

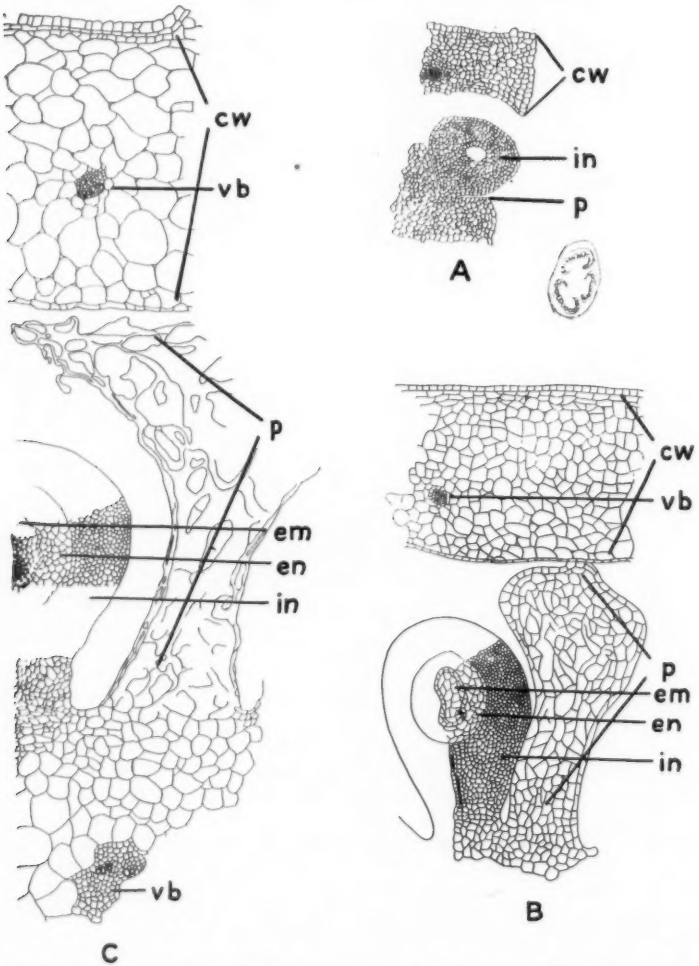

FIG. 5.—Camera-lucida drawings of sections of tomato fruits. × 66.3. A, fruit three days after fertilization; B, fruit approximately 10 days after fertilization; C, fruit ¾ inch to 1 inch in diameter. *cw*, carpel wall; *en*, endosperm; *em*, embryo; *in*, integument; *p*, placenta; *vb*, vascular bundle

Changes also occur in the seed as the fruit ripens. Souèges (*13*) has made the most extensive study of the development of the seeds of the family Solanaceae. The similarity in the development of the various members of this family is quite noticeable. This investi-

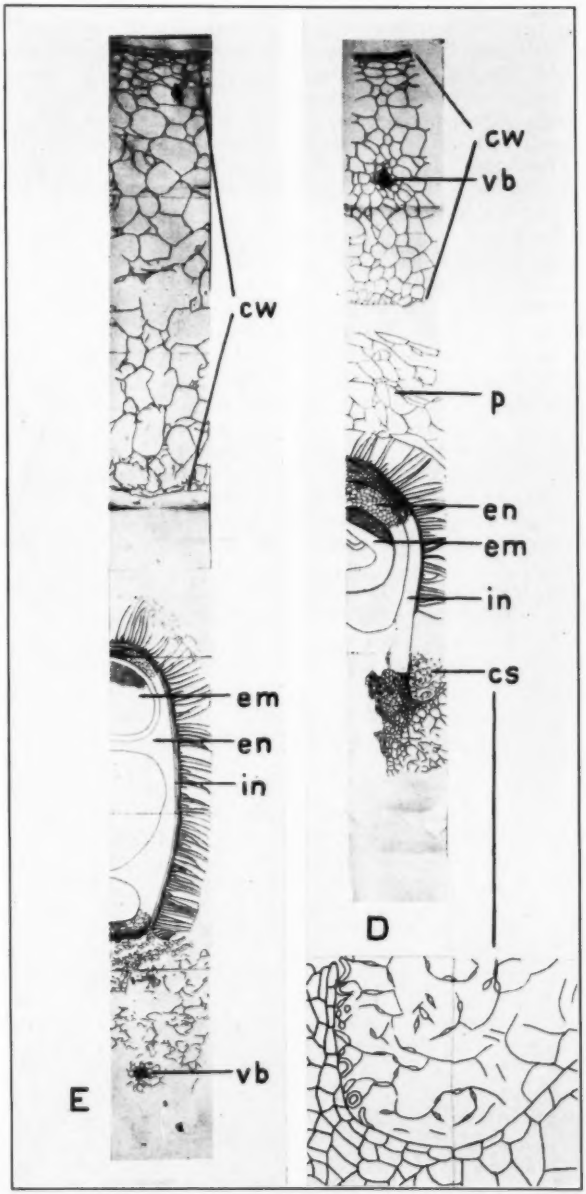

FIG. 6.—Camera-lucida drawings of sections of tomato fruits. × 23.6. D, green fruit before ripening; E, ripe fruit. *cs*, cross-section view of spines (enlarged below); *cw*, carpel wall; *en*, endosperm; *em*, embryo; *in*, integument; *p*, placenta; *vb*, vascular bundle

gator has studied the development of the macrogametophyte very carefully. Up to the time of fertilization the integument is composed of cells uniform in size and shape, but soon afterwards these differentiate into the external layer (assise externe) and the internal layer (assise interne) of the integument. Between these two layers are two regions called the internal and external zones (zone externe and zone interne). The nucellus is completely digested, so that the macrospore mother cell lies in direct contact with the internal layer of the integument. Some have considered this layer as the outermost layer of the nucellus.

The outer layer of integument cells enlarges as the seed matures and longitudinal thickenings of cellulose are formed in these walls. These thickenings increase in size, turn brown, and the intervening thin portions of the cell walls disintegrate, so that the thickenings appear to be spinelike. Figure 6, D shows on an enlarged scale the character of the cellulose thickenings from two aspects. The cross-section view near the funiculus shows where the thin cell walls have disappeared in some cells, and in other cells the thin wall can still be seen.

Tschirch and Oesterle (*15*) portray the same structures for the mature seed for Capsicum and Datura. In Datura the layer referred to by Souèges as the internal layer of the integument is called nucellus, while the same layer is referred to as the perisperm by Winton and Moeller (*18*) in their figure of the mature seed of *Lycopersicum esculentum*.

Groth (*4*) has made a study of the structure of the cells found in the "skin" of the fruit of various types of tomatoes.

DISCUSSION

The distribution of phosphorus in a starved plant and its distribution in a plant developed with an ample supply show striking similarities. The regions in which the highest percentages of phosphorus (based on dry weight) occur are the same, under both treatments (the fruit, cluster stems, top leaves, and top stem). In some earlier analyses of tomato plants grown in garden soil, in which the plants were more finely subdivided, it was found that the nodes and leaflets had a greater percentage of phosphorus than the adjacent regions—the internodes and midrib of the compound leaf. These facts seem proved by all the data, but especially by the percentages based on dry weight. The percentages of phosphorus in the plants receiving an ample supply of phosphorus show an almost steady increase from the bottom to the top regions of the stem. The plants grown in the nutrient solution lacking phosphorus do not show such an increase in phosphorus content, but approximately the same percentage of phosphorus is found in all the regions of the stem from the bottom to the next to top. This naturally leads to the conclusion that high percentages of phosphorus are associated with regions of rapid growth. In the plants grown in the absence of phosphorus the supply is so limited that only in the very top part of the plant is there active growth. It would be of interest to know just what the difference is between the organic and inorganic forms of phospohrus in such embryonic regions. If one could completely separate the embryonic regions for purposes of analysis it might be that these regions even

under different treatments would be found to have equal percentages of phosphorus, at least there might be less relative difference than these tables show.

As all the regions of high phosphorus content are areas which have developed subsequent to the plant's being transplanted to sand, there is no doubt that phosphorus is subject to reutilization during starvation or a time of limited supply. The regions in question are the fruit, cluster stems, top leaves, top stem, next-to-top leaves, and next-to-top stem. The total amount of phosphorus in all regions shows that, regardless of the treatment, approximately one-half of the total phosphorus is located in the fruit. The leaves in the bottom three regions should contain the same total amount of phosphorus at the end of the experiment as at the beginning if there is no reutilization of the element. The mere decrease in the percentage of phosphorus would not prove that the phosphorus had been removed, as the decrease in the percentage of phosphorus might be accompanied by an increase in the total dry weight. In experiment D, 68 per cent of the phosphorus has been removed from the next-to-bottom leaves, and in the same region in experiment F, 78 per cent has been removed. The leaves fell from the bottom region within a short time after transplanting—too soon to permit of the removal of such a large proportion of the phosphorus.

The nonremoval of phosphorus from these lower leaves might indicate that phosphorus was not transported with rapidity in the plant. Plants which had been phosphorus-starved for two months showed very slow recovery or nonrecovery when phosphorus was added to the nutrient solution. This is further evidence that the rate of movement of phosphates is dissimilar to that of nitrogen, as additions of nitrates can be recognized by plant performance within a few days.

The results obtained by André (*2*) show that phosphorus is redistributed to a greater extent than most of the other minerals. This property of great reutilization may be a contributing reason why plants have a smaller total amount of phosphorus than of nitrogen, potassium, and some other minerals. The results obtained by Le Clerc and Breazeale (*9*), Wilfarth, Römer, and Wimmer (*17*), Schulze and Schütz (*11*), and Wehmer (*16*) are suggestive of reutilization of the mineral elements, although the proof is not so conclusive as that presented by André.

Under the two treatments there were notable differences in the number of blossoms opened, the number of blossoms setting fruit, and the proportion of these fruits which continued to grow. Blossoms frequently turn yellow and fall from the plant before blooming. The data show that this is much more common in the plants grown in the absence of phosphorus than where ample phosphorus is present. Inasmuch as blossoms require cell division for their development it would seem as if the limited supply of phosphorus was not sufficient to provide the inorganic and organic phosphorus necessary. Even with the reutilization of this element the demands are not fulfilled.

As flower primordia are formed strikingly early in the life of a plant, it seems evident that these plants should have had the same number of flower buds, at least in the first few clusters. One should consider the possible effect of a limited supply of phosphorus at an early stage on the number of bud primordia formed. The phos-

phorus content of potting soils might thus influence the number of buds on the early clusters of a plant. When the possibilities of reutilization as well as the smallness of the quantities of phosphorus needed in such embryonic regions are taken into consideration, it seems unlikely that the number of floral buds would be reduced on this account.

The fruits which were immature at harvest were of two kinds: First, those which had made continuous growth; and secondly, those which had grown for only a short period after fertilization. It is likely that the cessation of growth in the latter was due to insufficient food supply. Single mature fruits were very prevalent on the plants grown in the absence of phosphorus; therefore it would seem that one fruit could cause a sufficient deficiency of phosphorus to prevent the development of the other fruits. The fruits which continued to develop were almost invariably the ones which were pollinated first. Priority of pollination perhaps accounts to a certain extent for the variation in the size of fruits within a single cluster. In ordinary plants grown in greenhouses some of the fruits of a cluster are larger than others. There is some variation in the position of the larger fruits, but usually they result from the earlier-pollinated flowers. Some of the flowers produce ovaries which develop only a few centimeters in diameter. Usually the calyx develops so as to accentuate the smallness of the ovary. A study of such factors as heredity, pollen, position of fruit in cluster, and position of cluster on plant, as affecting the size of the fruit, would be of interest.

It is commonly stated that phosphorus is essential to seed formation. Just what interpretation should be given to this statement is uncertain. Phosphorus is essential to the formation of cells and thus would be necessary to the formation of seed or any other structure of the plant, whether fruit or vegetative part. It is doubtful if phosphorus is mainly essential only to the formation of the seed portion of the fruit.

Such statements are probably based on analyses of the fruits of plants such as wheat and corn, which are commonly considered seeds, but the presence of the outer coat, which is a portion of the carpel wall, usually called a fruit coat, causes them to be classified as fruits. These fruits have rather a large amount of phosphorus, but just how much is in the seed is not known. Since the fruit coat of wheat is very thin and constitutes a very small percentage of the total wheat fruit, it seems likely that phosphorus might be more essential to its seed formation than to the seed formation of a tomato, the nonseed portion of the fruit of which has a dry weight more than double that of the seed portion. The tomato is not a very satisfactory fruit for determining whether the greatest amount of phosphorus is found in the seed, the carpel wall, or the placental pulp, because it is difficult to separate these portions from one another in a quantitative manner. However, even with this error which increases the amount of phosphorus in the portions classified as seed in this paper, there is some doubt as to the statement that phosphorus is essential only to seed formation. A plant such as a legume or some member of the cabbage family the fruit parts and seed parts of which are distinctly separate would be more desirable for this study.

An examination of Figure 3 shows that both treatments seem to produce normal seed. The germination tests and the growth of seeds in soil show that seeds from plants grown in the absence of phosphorus are able to reproduce the plant almost as efficiently as seeds from plants grown in the presence of an ample amount of phosphorus. The analyses based either on the percentage or on the total amount of phosphorus show that the seed suffers just as much as the pulp under limited phosphorus nutrient conditions.

Phosphorus is just as essential to the formation of pulp as to the formation of seed. The figures referred to in the previous paragraph indicate that the effects of phosphorus starvation on the development of pulp are as marked as its effects on the seed. The seed is mature and differs only in size; the pulp is decreased in amount both in the carpel walls and in the central placental region. The differences produced by the two treatments are of such great magnitude that one might suspect that the plants were of different varieties. A tomato whether grown in garden soil or in a nutrient solution containing an ample amount of phosphorus or a nutrient solution containing no phosphorus has a larger total quantity of this element in the pulp than in the seed. Of the total amount of phosphorus in a fruit, the percentage found in the seed varies little with treatment, being 29.3 per cent in the plants grown in the absence of phosphorus, and 35.7 per cent in the plants grown in the presence of an ample supply of phosphorus. It should be remembered, however, that the fruits produced by the no-phosphorus plants are smaller and have fewer seeds than those produced by the ample-phosphorus plants. A fruit grown in normal garden soil either with or without the applications of steamed bone meal has a greater total amount of phosphorus in the pulp than in the seed. This is because the pulp has a greater dry weight than the seed, and not because it has a greater percentage of phosphorus. This is true not only of ripe fruits, but also of all stages of green fruits in which it is possible to separate the seed and the pulp. The presence of phosphorus in such large quantities in the pulp at all times regardless of age or phosphorus nutrient treatment shows that phosphorus is essential not only to seed formation, but also to the formation of pulp.

It is of interest in connection with this discussion to consider some of the processes in the formation of fruit. The ripening process seems to include a change of carbohydrates of high molecular weight to sugars, an increase in dry and fresh weight, an enlargement of practically all cells, and the disintegration of the cell walls. The chemical changes during ripening have been very thoroughly investigated by Albahary (*1*) and Sando (*10*). The enlargement of the cells would not require large quantities of phosphorus, still the origin of such cells would require cell division in an embryonic region. Such a region would naturally be high in phosphorus whether it was to produce ovule or vegetative cells. After these cells are formed there is a great increase in their size, accompanied by an increase in the amount of phosphorus present. Thus maturation in the tomato is accompanied by progressive increases in the amount of phosphorus. The data in the ripening experiment show that the tissue contains the highest percentage of phosphorus in the embryonic stage. If tissue of an earlier stage could be obtained in sufficient quantities for

analysis, probably it would be found to contain a still higher percentage of phosphorus. The presence of an ample supply of phosphorus at such an early stage must have an important influence on the size of fruit which will develop. Some cell division must occur after the 3 to 5 mm. stage, as there are embryonic regions in these fruits and the placenta has not yet grown around the seed.

The weight of a single seed of a no-phosphorus plant is less than that of a single seed of a full nutrient solution plant. This result is in agreement with the results of similar experiments conducted by Hellriegel, Wilfarth, Römer, and Wimmer (*7*) with barley, in which the weight per seed increased with the amount of phosphorus added to the soil. Seeds from the no-phosphorus plants seem able to pass through the seedling stage and become independent plants. The lighter seeds seem to produce less stocky plants, a circumstance which might affect yield and earliness of fruit, as has been shown by previous workers with seeds of different weights. Seeds from plants grown under the two treatments when grown in sand cultures without phosphorus showed that the difference in phosphorus reserve affected the amount of growth.

Statements have been made to the effect that some plants can be supplied with sufficient phosphorus in the early stages of growth and are benefited by maturing in a solution lacking phosphorus (*3*). This investigation shows that a tomato plant can not absorb sufficient phosphorus from a greenhouse potting soil in eight weeks to supply its needs for continued development through an equal length of time without an additional supply of phosphorus. The effects of starvation were noticeable in some of the plants that had been deprived of phosphorus only two or three weeks. Gericke (*3*) worked with the wheat plant, which differs from the tomato in that it has a definite period of vegetation and a definite period of fruiting. In the tomato, fruit formation and production of vegetative growth may occur at the same time. As plants will absorb greater quantities of an element than they need for growth (*8*), it is likely that such excess of phosphorus plus the phosphorus removed from the old dying parts is sufficient to mature the fruit of the wheat in a nutrient solution lacking phosphorus.

Hartwell (*5*, *6*) has shown that plants differ as to their capacity to use the phosphorus of the soil. Carrots and tomatoes were able to obtain phosphorus from the soil for growth under conditions under which turnips, beets, and rape would be unable to grow satisfactorily without the addition of phosphate fertilizer.

The recovery of the phosphorus in the no-phosphorus plants was considered satisfactory, except in the case of the plants treated 24 days in experiment F. This large error may have been due to the greater amount of phosphorus in the newly formed roots and thus a greater loss in collecting this sample than there was in collecting the 64-day sample. It should be kept in mind that there were the following unavoidable very small errors in recovering the phosphorus: The errors of analysis; flower buds lost through abscission; loss of roots in removal from sand; and loss after blooming of such parts of the flower as the corolla, style, and stamens. There seems to have been no loss of phosphorus from the plant to the surrounding medium, which was much lower in phosphorus content.

In the absence of a sufficient amount of phosphorus one would expect a disorganization of the parts of the cell and consequently a disorganization of its normal functions. Cells in such condition are apparently unable to form chlorophyll to replace that lost through decomposition. Another circumstance which might explain chlorophyll deficiency is the accumulation of the end products of the reaction—sugars. As the accumulation of sugars would tend to stop the synthesis of sugars, it is unlikely that chlorophyll would be formed if it were not going to be used. These are possible explanations of the decreased chlorophyll content of the tomato leaves at the end of the experiment.

Some plants, such as the tomato, when grown under conditions of low nitrogen supply, develop certain marked external characteristics. Such plants are not infrequently spoken of as "nitrogen-starved" or as "carboyhydrate-high" plants. Because of the frequency with which these terms are used, it is easy to overlook the fact that a similar characteristic appearance may be produced by the deficiency of other essential elements or by other factors affecting growth. From the description of the plants under "Data and results," it would seem that phosphorus and nitrogen starvation have some external characteristics in common. Among these might be mentioned the stiffening of the stem; decrease in chlorophyll, as shown by yellow leaves; and the appearance on the under side of leaves of anthocyanin pigments.

The analyses giving the amounts of the elements in percentage show that embryonic regions, such as the fruit, cluster stem, top leaves, and top stem, are not only high in phosphorus, but also in nitrogen. The regions of highest nitrogen are, however, slightly different from the regions of highest phosphorus, being the top leaves instead of the fruit and cluster stems. As previously explained, these embryonic regions are high in protein, so one would expect a high percentage of the elements such as phosphorus and nitrogen, which compose the proteins. When a comparison is made of the percentages of nitrogen in the plants having had the two treatments the facts found are less easy to explain. Plants grown in sand in the absence of phosphorus usually have a greater percentage of nitrogen than plants having had an ample supply of phosphorus. The plants grown in the absence of phosphorus show a decrease in the percentage and also the total amount of coagulable nitrogen as compared with the total coagulable nitrogen in the plants grown in the presence of an ample supply of phosphorus. The increase in the percentage of nitrogen in the no-phosphorus plants is confined to the water-soluble forms of nitrogen, excluding nitrates.

Phosphorus starvation seems to affect the carbohydrates primarily as to the amount of sugars found in the plant, but its effect on the other carbohydrates is not very consistent. The quantity of reducing sugars and the total quantity of sugars increased greatly in the plants grown in the absence of phosphorus as compared with the quantities in the plants grown in the presence of an ample amount of phosphorus. These increases in sugars were usually accompanied by a lower percentage of starch. If, as has been previously shown, starvation interferes with the coagulable-nitrogen synthesis it is natural that there should be an increase in carbohydrates. It should be noted,

however, that phosphorus starvation and nitrogen starvation affect the tomato plant differently, as the latter brought about a predominant storage of starch.

The plants grown in the absence of phosphorus almost trebled their dry weight during both experiments. This seems in harmony with the increase in percentage of carbohydrates. It does not seem, however, that this would entirely account for this increase.. Some of this weight must have been due to cellulose and lignin substances not determined.

As experiment D was conducted in the fall and experiment F in the spring and as they were continued different lengths of time there were a number of variations in the results obtained. Thus, in experiment F there is a greater difference in the carbohydrates in the plants under the two treatments than is shown in experiment D. This might have been expected inasmuch as these plants were grown in the spring and the experiment extended over a longer period of time than did experiment D. The difference in the time of year and length of time the experiments were conducted seems to have exerted an almost negligible effect on the phosphorus content of the plants.

This study has been planned primarily to increase our knowledge of the effect of phosphorus starvation on the growth of the tomato plant, yet there are suggestions which are of practical significance.

Since the tomato is a plant which may normally have at the same time flowers and green fruit at various stages of maturity, it would seem essential to supply the plant with a continuous supply of phosphorus. This is necessary during fruit formation if good-sized fruits are desired. It is also advisable, inasmuch as about one-half of the phosphorus of the plant is found in the fruit. It should be possible to detect phosphorus-starvation conditions in the tomato by the symptoms here described.

SUMMARY

These studies show that phosphorus starvation has the following effects on a tomato plant: (1) The percentage of phosphorus is decreased in all parts of the plant; (2) there is a reutilization of the phosphorus; (3) regions of growth contain the highest percentage of phosphorus; (4) there is a decrease in the size of fruit; (5) the dry weight of the plant is trebled in two months; (6) the coagulable nitrogen content is decreased; (7) the percentage of total nitrogen increases, the increase being primarily in the water-soluble forms of nitrogen, excluding nitrates; (8) there is a marked increase in the percentage of sugars.

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CRITICAL TESTS OF TETRACHLORETHYLENE AS AN ANTHELMINTIC FOR FOXES[1]

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INTRODUCTION

In a recent paper Hall and Shillinger (*2*)[3] called attention to the promising results obtained by them in critical tests of tetrachlorethylene as an anthelmintic for dogs. They found the drug very effective against hookworms and ascarids. In a later paper these authors (*3*) published protocols of tests on about 60 dogs. Their experiments indicated that the effective dose was 0.2 c. c. per kilo. At this dose rate tetrachlorethylene removed 195 of 196 hookworms in 9 infested dogs and all the ascarids—32—in 4 infested dogs. Dogs survived doses of 3, 5, 10, and 15 c. c. per kilo, which indicates that the margin of safety is high in the absence of contraindications. Of 64 dogs treated with doses ranging from 0.2 to 0.6 c. c. per kilo, 3 died from 1 to 3 days after treatment. These three animals were showing clinical symptoms of distemper at the time of treatment. The authors concluded that the contraindications for tetrachlorethylene probably are identical with those for the related compound, carbon tetrachloride. Liver lesions were noticed after the administration of tetrachlorethylene.

Schlingman (*5*) performed critical tests of tetrachlorethylene on 31 dogs, mainly puppies 3 to 4 weeks old. In these tests the doses varied from 0.2 to 10 c. c. per kilo, and the drug was 100 per cent effective against hookworms in all cases and 87.7 per cent effective against ascarids in 25 cases. His findings indicate that the safety of tetrachlorethylene for puppies is higher than that of carbon tetrachloride. He also found that tetrachlorethylene apparently is not so likely to cause inhalation intoxication as is carbon tetrachloride when the containing capsules accidentally break in administration.

Since tetrachlorethylene has given promising results in tests on dogs, it was considered advisable to conduct critical tests of this new anthelmintic on foxes. The results of these experiments are reported in this paper. Of the 26 foxes used in these tests, 16 were pups from 7 to 9 months of age and the other 10 were 1 year old or more.

ANTHELMINTIC TESTS

In the experiments on foxes the animals were given chemically pure tetrachlorethylene after a fast of 16 to 20 hours. The drug was given in hard gelatin capsules, administered by means of a balling gun consisting of a rubber tube about 2 feet long and a rattan plunger.

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[2] The writer desires to acknowledge the cooperation given in the preparation of this paper by M. C. Hall and G. T. Creech, of the Bureau of Animal Industry, United States Department of Agriculture.

[3] Reference is made by number (italic) to "Literature cited," p. 136.

The mouth of the animal was restrained by means of a mouth clamp, an instrument consisting of a wooden gag and a clamping device for holding the jaws down tight on the gag. The rubber tube was passed three-quarters of the way down the esophagus and the capsules containing the drug were then forced down the tube by means of the plunger.

The animals were fed two to three hours after dosing, and while on the tests were given a ration once a day consisting of raw ground beef hearts and liver, baked cereals, whole milk, and bone meal

After treatment the foxes were confined in small individual cages with 1-inch-mesh wire bottoms, set over galvanized-iron collecting pans. All worms passed were collected once a day, identified, and counted. From 48 to 72 hours after dosing the animals were killed, either with an intracardial injection of strychnine sulphate or by stunning with a blow on the head, which was promptly twisted so as to dislocate the atlanto-occipital articulation. The worms present when the post-mortem examination was made were identified and counted, and gross pathological conditions were noted, especially those due to the anthelmintic.

Tests conducted to determine the anthelmintic efficiency of tetrachlorethylene when given without purgation are reported in Tables 1, 2, and 3.

TABLE 1.—*Anthelmintic efficiency of tretrachlorethylene given without purgation at a dose rate of 0.2 c. c. per kilo*

Fox No.	Weight	Number of worms passed		Number of worms found post-mortem		Efficacy in expelling—	
		Hookworms	Ascarids	Hookworms	Ascarids	Hookworms	Ascarids
	Kilos					*Per cent*	*Per cent*
1325	3.0	1	0	0	0	100	----------
1326	3.2	3	0	0	0	100	----------
1327	2.8	0	0	0	0	----------	----------
1328	2.8	20	3	0	0	100	100
1329	3.4	5	7	0	0	100	100
1330	3.2	27	0	2	0	93	----------
1337	4.4	5	0	0	0	100	----------
1338	4.2	0	0	0	0	----------	----------
1339	4.6	9	0	0	0	100	----------
1346	4.7	2	0	0	0	100	----------
1347	3.7	3	4	0	0	100	100
1348	3.0	10	1	0	0	100	100
Total	----------	85	15	2	0	97.7	100

All the foxes in the foregoing tests were constipated except Nos. 1337 and 1339, and after treatment these passed feces that were soft and semiliquid. Fox No. 1325 was showing marked clinical symptoms of lungworms when treated. On post-mortem a heavy infestation of *Capillaria aerophila* was found, and a chronic inflammation of the mucosa of the trachea, bronchi, and bronchioles, and in these parts there was an abundance of a very thick exudate.

TABLE 2.—*Anthelmintic efficiency of tetrachlorethylene given without purgation at a dose rate of 0.3 c. c. per kilo*

Fox No.	Weight	Number of worms passed		Number of worms found post-mortem		Efficacy in expelling—	
		Hookworms	Ascarids	Hookworms	Ascarids	Hookworms	Ascarids
	Kilos					*Per cent*	*Per cent*
1340	3.7	23	0	0	0	100	----------
1341	4.8	2	0	0	0	100	----------
1342	4.3	0	0	0	0	----------	----------
1343	4.1	18	1	0	0	100	100
1344	3.3	4	0	0	0	100	----------
1345	3.4	12	1	0	0	100	100
Total	----------	59	2	0	0	100	100

All the foxes were constipated except Nos. 1343 and 1345, the feces of which, passed after treatment, were soft and semiliquid.

TABLE 3.—*Anthelmintic efficiency of tetrachlorethylene given without purgation at a dose rate of 1 c. c. per kilo*

Fox No.	Weight	Number of worms passed		Number of worms found post-mortem		Efficacy in expelling—	
		Hookworms	Ascarids	Hookworms	Ascarids	Hookworms	Ascarids
	Kilos					*Per cent*	*Per cent*
1331	4.0	13	0	0	0	100	----------
1332	4.0	10	0	0	0	100	----------
1333	4.7	33	3	0	0	100	100
Total	----------	56	3	0	0	100	100

On all three foxes, records for which are given in Table 3, the tetrachlorethylene produced a laxative effect.

To determine whether the simultaneous administration of 1 grain of phenolphthalein along with tetrachlorethylene at a dose rate of 0.2 c. c. per kilo would affect the efficacy of the anthelmintic, tests were conducted, the results of which are summarized in Table 4.

TABLE. 4.—*Anthelmintic efficiency of tetrachlorethylene given at a dose rate of 0.2 c. c. per kilo together with 1 grain of phenolphthalein*

Fox No.	Weight	Number of worms passed		Number of worms found post-mortem		Efficacy in expelling—	
		Hookworms	Ascarids	Hookworms	Ascarids	Hook worms	Ascarids
	Kilos					*Per cent*	*Per cent*
1334	3.1	12	0	0	0	100.0	----------
1335	3.1	9	0	26	0	25.7	----------
1336	3.3	1	0	36	0	2.7	----------
Total	----------	22	0	62	0	26.1	----------

The phenolphthalein produced a laxative effect only in the case of fox No. 1335; the other two were constipated.

TOXICITY TESTS AND FINDINGS

In order to determine whether foxes are likely to suffer inhalation intoxication when tetrachlorethylene or its fumes are inhaled as a result of the accidental breaking of capsules during administration, six 1 c. c. capsules of the drug were broken in the mouth of each of two foxes. With the exception of a temporary irritation of the mouth, as evidenced by profuse salivation and a rubbing of the mouth with the paws, no apparent ill effects were produced by the drug. Under similar conditions the use of carbon tetrachloride would undoubtedly have brought about collapse and possibly death from inhalation intoxication.

To determine the effects of tetrachlorethylene administered in large doses by means of a stomach tube, the following tests were performed the next day on two foxes:

Fox No. 1349 (total dose, 30 c. c.; dose rate, 8.6 c. c. per kilo).—Vomited food given about 2 hours after dosing; manifested no other apparent symptoms; killed the eighth day after treatment.

Fox No. 1350 (total dose, 30 c. c.; dose rate, 7.5 c. c. per kilo).—Manifested no apparent symptoms; killed the eighth day after treatment.

To determine the microscopic changes produced in the liver by tetrachlorethylene, specimens were selected from the livers of 20 foxes, fixed either in 10 per cent formalin solution or Zenker's fluid, and sent to the Pathological Division of the Bureau of Animal Industry. In all cases the specimens were selected from those portions of the liver where evidences of pathological changes were most apparent macroscopically. Histological sections from these specimens were prepared and examined by Gilbert T. Creech, of the Bureau of Animal Industry. The findings in the livers of these foxes, as reported by Doctor Creech, are as follows:

Fox No. 1327 (dose rate, 0.2 c. c. per kilo).—The liver showed engorgement of the central veins, with distention of adjacent capillaries. The liver cells as a whole presented a pale or granular appearance, and certain of the lobules, particularly those toward the central veins, contained cells in varying stages of degeneration. Many of the cells showed a loss of nuclei, and some had completely atrophied. A few necrotic foci were seen in this specimen, and a few areas of round-cell infiltration were also noted. There was a limited amount of edema present.

Fox No. 1328 (dose rate, 0.2 c. c. per kilo).—The liver showed slight venous and capillary engorgement. The changes were somewhat similar to those noted in the liver of fox No. 1327. Degeneration of the liver cells was evidenced by cloudiness and the loss of nuclei. Vacuolation of the liver cells was quite noticeable in this case.

Fox No. 1329 (dose rate, 0.2 c. c. per kilo).—The liver contained considerable blood, mostly congested in the capillaries. There was marked edema in this case. The liver cells were in stages of degeneration varying from a slight cloudiness of the chromatin to complete atrophy. Vacuolation of the liver cells was also noted.

Fox No. 1332 (dose rate, 1 c. c. per kilo).—The liver was acutely venous and displayed capillary congestion, with small hemorrhages and also a limited amount of edema. Degeneration and atrophy of the cells were noted, particularly in the region of the central veins. Coagulated blood was seen in a number of the vessels, and several small areas of round-cell infiltration were noted.

Foxes Nos. 1334 and 1336 (dose rate, 0.2 c. c. per kilo and 1 grain phenolphthalein).—A number of the liver lobules showed venous and capillary engorgement. Perivascular cellular infiltration was noted in several places. A large percentage of the liver cells showed degenerative changes, and in certain lobules columns of liver cells had undergone complete atrophy. Vacuolation of the liver cells was seen throughout the section. There was rather pronounced edema in this case.

Fox No. 1337 (dose rate, 0.2 c. c. per kilo).—The capillaries toward the center of the liver lobe were engorged with red cells, which stain very poorly. There appeared to have been more or less of a blood stasis in portions of this liver. A number of the larger vessels contained thrombi. A large proportion of the liver cells appeared to be undergoing degenerative changes. In fact there were few normal cells to be seen. An organized exudate was seen surrounding the walls of a number of the vessels.

Foxes Nos. 1338 and 1339 (dose rate, 0.2 c. c. per kilo).—The blood changes in these livers were slight. The specimens showed a noticeably edematous condition. Practically all the liver cells showed cloudiness, and many a loss of nuclei. A number of irregular spaces were seen in which the liver cells had undergone complete atrophy.

Fox No. 1340 (dose rate, 0.3 c. c. per kilo).—The liver showed a passive congestion, and there appeared to have been a blood stasis in the central portion of the lobe. A number of the large vessels showed thrombic formations. The liver cells showed advanced degenerative changes, and in places rather large areas of cell necrosis were seen. In a number of places cellular infiltrations of the interstitial tissue were noted. The capillary engorgement and degenerative process were less marked toward the periphery of the organ.

Fox No. 1341 (dose rate, 0.3 c. c. per kilo).—There had been considerable circulatory disturbance in the liver. The large vessels contained thrombi, and there was capillary engorgement, particularly toward the central portion of the lobe. An organized exudate was seen surrounding many of the larger blood vessels. The degenerative changes in the cells were quite similar to those in the liver of fox No. 1340. A limited amount of perivascular cellular infiltration was seen in this specimen.

Foxes Nos. 1342, 1343, 1344, and 1345 (dose rate, 0.3 c. c. per kilo).—The changes in the livers, consisting of thrombic formations in the larger vessels, capillary blood stasis, and degenerative and atrophic changes in the liver cells, simulated rather closely those described in the livers of foxes Nos. 1340 and 1341.

Fox No. 1346 (dose rate, 0.2 c. c. per kilo).—Circulatory disturbance was noticeable in this case. The larger vessels showed thrombic formations, and the capillaries in the central lobules were distended with degenerated erythrocytes. Edema with coagulated exudate could be seen surrounding many of the larger vessels. There was marked cloudy swelling of the liver cells, the degenerative and atrophic changes being in evidence throughout the sections examined.

Fox No. 1347 (dose rate, 0.2 c. c. per kilo).—The changes were similar to those noted in the liver of fox No. 1346.

Fox No. 1348 (dose rate, 0.2 c. c. per kilo).—The circulatory disturbance and degenerative changes in the liver cells simulated those seen in the livers of foxes Nos. 1346 and 1347. The vacuolation of the liver cells was somewhat more pronounced in this specimen, and a number of small necrotic foci were noted, a few of which showed round-cell invasion.

Fox No. 1349 (dose rate, 8.6 c. c. per kilo).—The capillaries in the central portion of the liver were engorged with degenerated red cells, giving an appearance of blood stasis. Many of the larger vessels contained thrombi. Advanced degenerative changes in the liver cells were seen in this specimen, and areas of cell necrosis were seen throughout the sections examined.

Fox No. 1350 (dose rate, 7.5 c. c. per kilo).—The histological changes in the liver closely simulated those described in connection with the liver of fox No. 1349.

DISCUSSION

The anthelmintic efficiency of the various dose rates of tetrachlorethylene used in the foregoing tests may be summarized as follows:

A 0.2 c. c. per kilo dose removed 85 hookworms from 10 infested foxes and left 2, an efficacy of 97.7 per cent, and removed 15 ascarids from 4 infested foxes and left none, an efficacy of 100 per cent,

A 0.3 c. c. per kilo dose removed 59 hookworms from 5 infested foxes and left none, an efficacy of 100 per cent, and removed 2 ascarids from 2 infested foxes and left none, an efficacy of 100 per cent.

A 1.0 c. c. per kilo dose removed 56 hookworms from 3 infested foxes and left none, an efficacy of 100 per cent.

A 0.2 c. c. per kilo dose, *together with 1 grain of phenolphthalein* removed 22 hookworms from 3 infested foxes and left 62, an efficacy of 26.1 per cent.

In agreement with the findings of Hall and Shillinger (*3*), and also those of Schlingman (*5*), the results of these experiments indicate that tetrachlorethylene without the simultaneous administration of a purgative is highly effective against hookworms and apparently very effective against ascarids.

Since the foregoing experiments on foxes were performed, a paper has reached the author in which Schlingman (*6*) reports tests of tetrachlorethylene on seven foxes and other animals. In his tests the doses of the drug used on foxes ranged from 0.26 c. c. to approximately 3.0 c. c. per kilo. In all cases where the foxes were infested, the drug was found 100 per cent effective against both hookworms and ascarids.

A comparison should be made of the anthelmintic efficacy of tetrachlorethylene and carbon tetrachloride, heretofore the best-known drug for the treatment of foxes for the removal of hookworms. In the foregoing tests tetrachlorethylene alone at the indicated dose rate of 0.2 c. c. per kilo was 97.7 per cent effective against hookworms, removing 85 of the 87 hookworms from 10 infested foxes, and was 100 per cent effective against ascarids, removing all 15 ascarids from 4 infested foxes.

In similar tests of carbon tetrachloride alone at a dose rate of 0.2 c. c. per kilo, Hanson and Van Volkenberg (*4*) found the drug 97.7 per cent effective against hookworms, removing 44 of the 45 hookworms from four infested foxes, and 89 per cent effective against ascarids, removing 24 of the 27 ascarids from three infested foxes.

On the basis of this comparison the two drugs are about equally effective in the removal of hookworms from foxes, and tetrachlorethylene apparently is more effective than carbon tetrachloride in the removal of ascarids. This is in keeping with the conclusions reached by Hall and Shillinger (*3*) on the basis of a similar comparison of tests on dogs.

The simultaneous administration of phenolphthalein caused the efficacy of the indicated dose (0.2 c. c. per kilo) of tetrachlorethylene against hookworms to drop from 97.7 per cent to 26.1 per cent. This result also is in keeping with the findings of Hall and Shillinger (*3*), who found that the effectiveness of tetrachlorethylene was diminished by the simultaneous administration of a purgative.

Tetrachlorethylene, in contrast with carbon tetrachloride, apparently is not attended by danger of inhalation intoxication, as is indicated by the fact that breaking several capsules of the drug in the mouths of two foxes failed to produce symptoms of this type of intoxication. Schlingman (*5*) concluded that the difference in the effects of the two drugs in this respect is due to the fact that tetrachlorethylene is considerably less volatile than carbon tetrachloride.

None of the 26 foxes used in the foregoing tests died from effects of treatment. The only animal (fox No. 1349) to manifest untoward symptoms was one given the enormous dose of 8.6 per kilo. This animal vomited the first meal fed after dosing, but showed no other ill effects. The fact that five of the foxes tolerated doses greatly in excess of the indicated dose of 0.2 c. c. per kilo, these animals

surviving doses ranging from 1 c. c. to 8.6 c. c. per kilo, indicates that the margin of safety for tetrachlorethylene is very high in the absence of contraindications. This finding is in agreement with the findings of Hall and Shillinger (*3*) and Schlingman (*5*) in tests on dogs and with those of Schlingman (*6*) in tests on foxes.

The gross pathological changes which apparently were due to tetrachlorethylene seemed to be restricted to the liver. Some of the livers gave evidence of degenerative changes and lobular hemorrhage. Macroscopically the changes appeared to be irregularly distributed, some portions of the liver being affected more than others.

In spite of the fact that all the 26 foxes used in the experiments survived treatment with tetrachlorethylene and only 1 manifested apparent symptoms (the animal that had had the enormous dose of 43 times the effective dose), microscopic examination of the livers of 20 of the foxes indicated that tetrachlorethylene caused liver injury. The injury occurred even in cases where a dose of 0.2 c. c. per kilo was used. In some of the livers a necrosis of the cells was noted. The histological findings indicate that it would be advisable to give a thorough purgative about one hour after the administration of tetrachlorethylene. Such treatment ought to diminish systemic absorption of the anthelmintic and thereby decrease the extent of liver injury. In cases where the tetrachlorethylene is given in soft elastic globules, it would be advisable to defer the administration of the purgative until about two hours after the globules are given. The findings of Schlingman (*6*) in tests on dogs indicate that there is a loss of anthelmintic efficiency when tetrachlorethylene in soft elastic globules is followed in less than two hours by dry Epsom salts.

The observations made in the foregoing experiments indicate that phenolphthalein in a dose of 1 grain is not sufficiently effective as a purgative to use in conjunction with tetrachlorethylene in the treatment of foxes. This drug failed to produce a laxative effect in two of the three foxes to which it was given. Undoubtedly either Epsom salts or castor oil would be more satisfactory than phenolphthalein.

On the basis of the findings of Schlingman (*5*) in tests on dog puppies, it might be inferred that young nursing fox puppies may tolerate treament with the effective doses of tetrachlorethylene. On this point conclusions should be withheld, however, until sufficient investigation has been carried on to determine the tolerance of nursing fox pups to tetrachlorethylene.

Hall and Shillinger (*3*) state that "the contraindications for tetrachlorethylene will probably be identical with those for carbon tetrachlorid." Among the probable contraindications they list the following: Febrile and debilitating diseases, extreme youth or old age, gastroenteritis, liver lesions, prolonged fasting, diets of fats, oils, and cream, and simultaneous administration of alcohol.

Davis (*1*), in experiments on dogs, found that factors of diet exerted an enormous influence upon the toxicity of carbon tetrachloride. It is quite probable that factors of diet will exert a similar influence on the toxic ty of the related compound, tetrachlorethylene. In summarizing his experiments regarding the influence of diet upon the liver injury produced by carbon tetrachloride, Davis states that "Mixed diets and high protein diets—which may be considered normal

for dogs—are more or less protective. High carbohydrate diets afford very striking protection against liver injury. Starvation, on the other hand, is harmful. 'All fat' diets preceding carbon tetrachloride administration are conducive to a maximal injury of liver parenchyma."

CONCLUSIONS

Tetrachlorethy'ene is as effective as carbon tetrachloride in the removal of hookworms from foxes, and apparently more so in the removal of ascarids. The indicated dose is 0.2 c. c. per kilo, or 1 c. c. per 11 pounds, making 1 c. c. about the proper dose for the average-sized adult fox.

In contrast with carbon tetrachloride, in cases when the containing capsules accidently break, the administration of tetrachlorethylene apparently is not attended with the danger of inhalation intoxication.

The contraindications for tetrachlorethylene probably are the same as those for carbon tetrachloride. In the absence of contraindications, the safety factor for tetrachlorethylene is very high.

Tetrachlorethylene, even in therapeutic doses, may produce liver injury.

The simultaneous administration of a purgative or laxative diminishes the efficacy of tetrachlorethylene. It is advisable to give an effective purgative one to two hours after administration, as so doing probably lessens intestinal absorption of the anthelmintic and diminishes the extent of liver injury.

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EFFECT OF CARBON DIOXIDE UPON THE GERMINATION OF CHLAMYDOSPORES OF USTILAGO ZEAE (BECKM.) UNG.[1]

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INTRODUCTION

Much interest has been shown during the past few years in the stimulating effect of various plant tissues upon the germination of spores. Not only has this stimulation occurred when the plant tissue was in contact with the spores or present in the culture medium, but, as has been shown by experiments, some volatile substance from the plant tissue strongly affects spore germination.

Brown (*3*)[2] first demonstrated this stimulating effect upon the germinating spores of *Botrytis cinerea*, *Botrytis parasitica* (*allii*), *Monila fructigena*, and *Penicillium glaucum* by placing them in Petri dishes containing certain plant tissues. He concluded:

> The germination of *Botrytis cinerea* spores is increased by the action of volatile substances arising from certain plant tissues, such as apple leaves and fruit, leaves of *Ruta*, *Eucalyptus*, etc.

He stated further:

> It was shown in earlier papers of this series that the conditions present in the infection drop are in general those of feeble nutrition. The vigor of germination in the infection drop—a factor of primary importance in deciding whether attack is possible or not—has been shown in No. VIII of the present series to be influenced by the passive exosmosis of food substances from the host tissue into the infection drop. The present results show that, granted suitable conditions, a like effect can be produced through the action of volatile substances arising from the plant and accumulating in the atmosphere.

As is evident from the above quotation, Brown considered that the stimulating effect of the volatile organic substances arising from certain plant tissues was a nutritive effect upon the spores, thereby producing an increased "vigour of germination." It must be remembered, however, that in determining the amount of germination, he measured a large number of germ tubes and divided the total length of the germ tubes by the number of spores counted. This is a confusion of germination, with growth after germination Growth after germination is not always an exact index of the amount of germination that has taken place. For instance, 10 germinated spores out of a possible 100 may, by increased growth of their germ tubes, obtain the same average germ-tube length as 20 germinated spores out of a possible 100 under conditions less favorable for growth. In the latter case, however, the percentage of germination would be twice as great as that in the former case.

Noble (*10*), working with *Urocystis tritici* Koern., obtained a stimulatory effect by germinating the spores in the presence of "un-

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[2] Reference is made by number (italic) to "Literature cited," p. 147.

injured seedlings of nonsusceptible plants (field peas, beans, and rye)." He concluded:

> Such stimulation might be expected in the field when the environment causes a certain amount of anaerobic respiration of the plant roots.

He also obtained a stimulatory effect by placing the spores in distilled water in Syracuse dishes and putting them in a vessel containing a small amount of "expressed sap of wheat seedlings." His conclusion as to the stimulating effect in that instance was that—

> It is possible in these cases that the stimulus may have been derived from some of the original constituents of the plant sap, or it may have been derived from some of the products of its decomposition, possibly organic acids, esters, or alcohols.

He correlated the stimulating effect obtained in the above experiments with stimulation obtained by the use of such organic substances as benzaldehyde, salicylaldehyde, butyric acid, and acetone. His suggestion regarding the action of the stimuli is "that the action of the stimulatory agents is mainly such as to cause a change in the physical condition of the protoplasmic spore contents, and thus increase the permeability of the latter."

Durrell (*6*) found that, when certain plant tissues were placed in receptacles containing spores of *Basisporium gallarum* (out of contact with the germination medium), a stimulating effect on germination was produced. This stimulating effect, however, was not obtained when the experiment was conducted in the presence of a barium hydroxide solution. He concluded that the stimulation was due to the carbon dioxide given off by the plant tissues and found that a like stimulation could be obtained by placing the spores in an atmosphere of from 1 to 5 per cent of carbon dioxide.

The above-mentioned investigators, using the spores of various fungi, have definitely shown a stimulatory effect of plant tissue and of carbon dioxide. However, they have not explained the mechanism of this action The work of Brown (*2*) on the inhibiting effect of carbon dioxide on fungal growth has cast some doubt as to the possibility of any stimulating effect of carbon dioxide when used in such large quantities as were used by Durrell in his studies of *Basisporium gallarum*. Further research therefore seemed advisable to determine the real effect of plant tissue and carbon dioxide. It is the purpose of the following study, using the spores of *Ustilago zeae*, to show in more detail the effect of plant tissue and carbon dioxide upon spore germination.

EFFECT OF PLANT TISSUE ON THE GERMINATION OF SPORES OF USTILAGO ZEAE

It is not known to what extent a stimulatory effect upon spore germination is a general characteristic of living plant tissue. In order to demonstrate this, tests were made with 20 different plant tissues, a greater variety than was used by the above-mentioned investigators.

In these tests a 3 per cent solution of agar was poured into sterilized Petri dishes. After the agar had cooled, microscope slides on which were placed the solid tissues used, or small vials containing the liquid substances used, were placed in the dishes upon the agar. The spores were then dusted over the remaining surface of the agar and were, therefore, not in contact with the plant tissue (fig. 1). The cultures

were kept at a temperature of 30° C., which had been found by Jones (*8*) to be optimum for the germination of the spores of *Ustilago zeae*.

In determining the amount of germination, three representative fields in the dishes were selected, each containing from 75 to 100 spores. The average number of germinated spores in the three fields expressed as a percentage of the entire number of spores in the three fields was considered a fair index of the amount of germination in each case.

Table 1 shows the results of 10 trials in which readings on the percentage of germination and the production of sporidia were made at intervals of 3, 6, 12, and 24 hours.

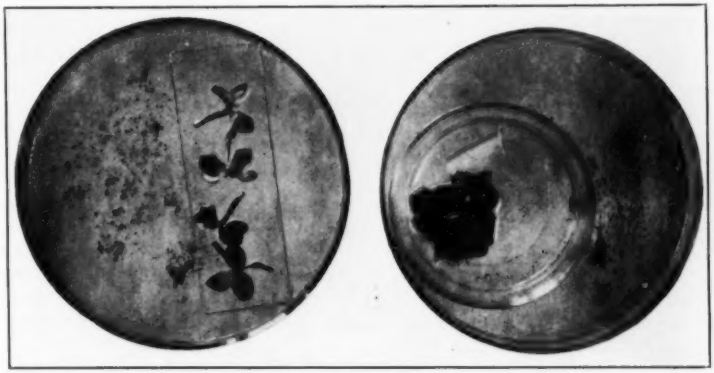

FIG. 1.—Petri dishes containing agar cultures of chlamydospores and plant tissue used in preliminary stimulation studies

TABLE 1.—*Stimulating effects of plant tissue upon the germination of the chlamydospores of Ustilago zeae*

Plant tissue used as stimulant	Results after 3 hours		Results after 6 hours		Results after 12 hours		Results after 24 hours	
	Percentage of germination	Production of sporidia	Percentage of germination	Production of sporidia	Percentage of germination	Production of sporidia	Percentage of germination	Production of sporidia
Control	None	None	Trace.	None	2.6	None	43.1	Fair.
Sweet pea petal	Trace	do	1.4	do	9.2	Trace	60.0	Very abundant.
Poppy petal	do	do	1.8	do	6.8	do	60.3	Do.
Rose petal	do	do	2.2	do	12.5	Fair	84.2	Do.
Lilac blossom	do	do	2.2	do	14.2	do	64.5	Abundant.
Spirea blossom	do	do	3.3	Trace	28.3	do	86.5	Very abundant.
Apple leaf	do	do	2.2	None	6.2	Trace	60.8	Abundant.
Geranium leaf	do	do	3.8	Trace	20.0	Fair	85.8	Very abundant.
Corn leaf	do	do	1.4	None	5.6	Trace	56.5	Do.
Rhubarb petiole	do	do	1.6	Trace	12.5	do	80.8	Do.
Pine needles	None	do	1.5	None	6.5	do	62.8	Do.
Dill	do	do	Trace.	do	6.3	do	59.5	Fair.
Green tomato	Trace	do	3.3	Trace	19.2	Fair	84.3	Very abundant.
Ripe tomato	do	do	3.0	do	28.2	do	86.5	Do.
Apple peel	None	do	Trace.	None	9.5	Trace	80.6	Abundant.
Apple pulp	Trace	do	1.9	do	11.3	do	80.3	Do.
Orange peel	do	do	2.5	do	15.3	do	82.3	Do.
Orange pulp	do	do	2.5	do	12.5	do	81.0	Do.
Lemon peel	do	do	2.5	do	17.2	do	82.3	Do.
Lemon pulp	do	do	1.8	do	15.5	do	72.3	Do.
Strawberry	do	do	1.2	do	7.5	do	69.8	Do.

It may be seen from Table 1 that all the substances used had a stimulating effect upon the germination of the spores. The stimulation is manifest not only in the percentage of germination but also in the character of the germination, a greater production of sporidia being characteristic of the stimulated cultures. Moreover, in the cultures containing plant tissue the spores germinated several hours earlier than in the control cultures. This increase in the speed of germination correlates well with the subsequent production of abundant sporidia. The point of greatest interest, however, is the fact that such a variety of plant tissues stimulate spore germination. This seems to preclude the theory that the stimulation is due to the action of specific volatile substances peculiar to certain plant tissues, and suggests that it is due to some volatile substance universally given off by plant tissues.

RELATION OF CARBON DIOXIDE TO THE STIMULATORY ACTION OF PLANT TISSUE ON SPORE GERMINATION

Tashiro (*12, p. 11*) found that crushed or bruised plant tissue liberates a large amount of carbon dioxide in a very short time. In order to demonstrate more fully that carbon dioxide given off by plant tissue has a stimulatory action on spore germination, the spores of *Ustilago zeae* were dusted on a 3 per cent nonnutient solution of agar in Syracuse dishes which were subsequently placed in air-tight moist chambers. The plant tissues (crushed orange or shredded green corn leaves) were placed in open dishes set in the moist chambers. A saturated solution of barium hydroxide (100 c. c.) was poured into some of the moist chambers to absorb any carbon dioxide that might be given off by the plant tissue.

In another series of experiments, similar moist chambers were exhausted to a partial vacuum and allowed to fill with air that had passed through a flask containing the plant tissue and then through three wash bottles of the barium hydroxide solution. A subsequent constant flow of air from the plant-tissue chamber through the barium hydroxide and into the germination chambers was maintained by an aspirator. A parallel series of experiments was conducted, which was similar to the above in every detail except that the spores were dusted into dry, clean Syracuse dishes instead of onto moist agar.

As controls in the above experiments, some moist chambers were used which contained only water to produce a humid atmosphere and some which contained only the plant tissue. The cultures were kept at a temperature of 30° C., and the germination counts were made after 24 hours. A summary of the results obtained in 10 trials is given in Table 2.

It will be noted in Table 2 that in the control cultures of agar containing no plant tissue a germination of 42.8 per cent was obtained. Cultures containing 75 to 100 grams of crushed orange germinated 91.8 per cent, while similar cultures in the presence of 100 to 150 grams of shredded corn leaves germinated 83.2 per cent.

In the presence of barium hydroxide a germination comparable to the control cultures containing no plant tissue was obtained. The control cultures on dry glass surfaces containing no plant tissue showed a trace of germination. When plant tissue was placed in the chambers containing the cultures on dry glass surfaces about 10 per

cent germination resulted. Here again, in the presence of barium hydroxide, a germination comparable to the control cultures containing no plant tissue was obtained. The water of condensation collecting on the spores dusted on dry glass surfaces seemed sufficient to produce the germination of some of the spores. This water could be readily seen through the microscope. In general, the results shown in Table 2 indicate that the stimulatory agent affecting spore germination in the presence of plant tissue is carbon dioxide.

TABLE 2.—*Inhibitory effect of barium hydroxide upon the stimulation of spore germination by plant tissue*

Medium	Modification of environment	Percentage of germination
Agar	Control (no plant tissue)	42.8
Do.	Control (75 gm. to 100 gm. of crushed orange)	91.8
Do.	Control (100 gm. to 150 gm. of shredded corn leaves)	83.2
Do.	Crushed orange and barium hydroxide	51.7
Do.	Shredded corn leaves and barium hydroxide	48.3
Do.	Air passed over crushed orange and through barium hydroxide	44.3
Do.	Air passed over shredded corn leaves and through barium hydroxide	39.6
Glass surface	Control (no plant tissue)	Trace.
Do.	Control (75 gm. to 100 gm. of crushed orange)	11.4
Do.	Control (100 gm. to 150 gm. shredded corn leaves)	9.8
Do.	Crushed orange and barium hydroxide	6.1
Do.	Shredded corn leaves and barium hydroxide	4.2
Do.	Air passed over crushed orange and through barium hydroxide	Trace.
Do.	Air passed over shredded corn leaves and through barium hydroxide	Trace.

QUANTITY OF CARBON DIOXIDE PRODUCED BY PLANT TISSUE

Durrell (*6*) in his work gave little consideration to the quantity of carbon dioxide given off by the plant tissues used. A series of experiments was therefore conducted by the present writers to determine the quantity of carbon dioxide produced by plant tissue in amounts comparable to those used in the above germination tests. It has been previously noted that from 75 to 100 grams of crushed orange and from 100 to 150 grams of shredded corn leaves produced the best stimulatory effect in moist chambers with a capacity of 2 liters. The quantity of carbon dioxide given off by these tissues was determined by passing air free from carbon dioxide into chambers containing the tissues and then into a saturated solution of barium hydroxide. A constant flow of air through the apparatus, maintained by means of an aspirator, produced a heavy precipitate of barium carbonate, which was measured by titration, as described by Harter and Weimer (*7*). In these experiments atmospheres containing from 10 to 15 per cent of carbon dioxide were obtained in the culture chambers.

ACIDITY IN SPORE-CULTURE MEDIA PRODUCED BY CARBON DIOXIDE FROM PLANT TISSUE

In view of the fact that water absorbs carbon dioxide very readily, thereby increasing the acidity of the solvent, and that acidity markedly affects cell permeability, it seemed logical to analyze the stimulatory effect of carbon dioxide upon spore germination by determining the change of hydrogen-ion concentration produced in the culture media by the presence of plant tissue.

The apparatus used in this study is shown in Figure 2. Drops of media containing suitable indicators were suspended in small vials containing bits of plant tissue such as tomato pulp, orange pulp, apple pulp, green corn leaves, wheat seedlings, and corn seedlings. As controls, drops were suspended in other vials each containing a small amount of barium hydroxide solution. The drops were suspended by drawing the liquid media containing the indicators into glass pipettes inserted through the corks of the vials. The tops of the pipettes were plugged with plasticene. Pressure on the soft plasticene plugs caused the colored media to exude in drops from the

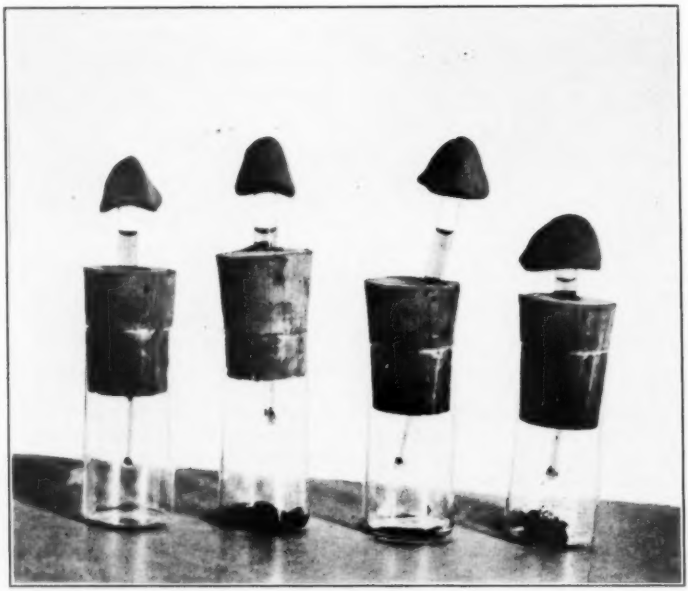

FIG. 2.—Drops (containing indicators) suspended in chambers above fragments of plant tissue

end of the pipettes within the vials. Conditions herein described are comparable to hanging-drop cultures in Van Tieghem cells. The vials were kept at a constant temperature for 24 hours and were then compared with standard indicators (*5*). The results of this experiment are shown in Table 3.

TABLE 3.—*Effect of the presence of plant tissue upon the hydrogen-ion concentration of media*

Medium	Atmospheric conditions	P_H at beginning of experiment	P_H at end of experiment
Tap water	Control (air over $Ba(OH)_2$)	6.7	6.7
Do	Control (air only)	6.7	5.5
Do	Tomato tissue present	6.7	4.9
Do	Orange tissue present	6.7	4.9
Do	Apple tissue present	6.7	4.9
Do	Wheat seedlings present	6.7	4.9
Do	Corn seedlings present	6.7	4.9

As is evident from Table 3, carbon dioxide given off by the plant tissue changed the acidity of the media in the drops from 6.7 P_H to approximately 4.9 P_H. It was difficult to make these determinations accurately, since refraction of light affected the color of the drops. The results, however, plainly indicate that the amount of carbon dioxide given off by plant tissue and absorbed by the drops was sufficient to change the acid reaction.

In order to obtain more accurate data, 5 c. c. of the medium (tap water, a 3 per cent agar solution or a 10 per cent gelatin solution) containing suitable indicators were poured into each of several 125 c. c. bottles of the same interior diameter as the Syracuse dishes used in the germination studies. The air was first exhausted from these bottles by means of a vacuum pump. They were then filled with carbon dioxide and air in varying proportions. Atmospheres containing 5, 10, 15, 20, 25, and 30 per cent of carbon dioxide were used. The bottles were kept at a temperature of 30° C. for 24 hours (conditions maintained in the germination tests). Results of 10 trials in the above experimental work are summarized in Table 4.

TABLE 4.—*The effect of CO_2 upon the P_H of media*

Medium	Percentage of CO_2 used	P_H at beginning of experiment	P_H at end of experiment
Tap water	5	6.5	5.40
Do	10	6.5	5.20
Do	15	6.5	4.95
Do	20	6.5	4.85
Do	25	6.5	4.75
Do	30	6.5	4.65
3 per cent agar	5	6.5	6.40
Do	10	6.5	5.80
Do	15	6.5	5.83
Do	20	6.5	5.10
Do	25	6.5	4.95
Do	30	6.5	4.85
10 per cent gelatin	5	6.7	6.30
Do	10	6.7	5.90
Do	15	6.7	5.65
Do	20	6.7	5.25
Do	25	6.7	5.10
Do	30	6.7	4.95

In Table 4 it may be noted that the hydrogen-ion concentration of the media is appreciably changed by atmospheres containing from 5 to 30 per cent of carbon dioxide. Atmospheres containing 15 per cent of carbon dioxide, such as had been found favorable for the germination of the spores of *Ustilago zeae* in the presence of plant tissue, changed the hydrogen-ion concentration of tap water from 6.5 to 4.9. The acidity change of the colloidal substances was not as great. The P_H of agar was changed from 6.5 to 5.3 and of gelatin from 6.7 to 5.6. This is in accordance with the findings of Cornelia Lee Carey (*4*), who showed that the intake of carbon dioxide in colloidal substances is proportional to the water content, the water acting as a medium for the absorption of this gas.

THE RELATION OF THE ACIDITY CHANGE PRODUCED BY CARBON DIOXIDE TO SPORE GERMINATION

To further correlate the above findings with the results of germination studies, *Ustilago zeae* spores were germinated in atmospheres containing various quantities of carbon dioxide. In these tests, the results of which are tabulated in Table 5, the air was exhausted from the culture chambers and replaced with atmospheres containing the following percentages of carbon dioxide: 5, 10, 15, 20, 25, 30, 50, and 75. Within the chambers were placed Syracuse dishes containing the media on which the spores were dusted. In order to eliminate stimulation from any foreign substances in the media used, nonnutrient media were chosen. Tap water, a 3 per cent solution of washed agar, a 10 per cent solution of gelatin, a silica jelly, made as Smith (*11, p. 36*) directs, and collodion films, made as Walpole (*13*) suggests, were used. The culture chambers were kept at a temperature of 30° C., and germination counts, previously described, were made after 24 hours.

TABLE 5.—*Stimulating effects of CO_2 upon the germination of the chlamydospores of Ustilago zeae*

Medium	Percentage of CO_2 used	P_H at beginning of experiment	P_H at end of experiment	Percentage of germination (after 24 hours)
Tap water	Control.	6.5	6.50	0.0
Do	5	6.5	5.40	Trace.
Do	10	6.5	5.20	2.0
Do	15	6.5	4.95	6.0
Do	20	6.5	4.85	2.0
Do	25	6.5	4.75	Trace.
Do	30	6.5	4.65	Trace.
Do	50	6.5	4.40	0.0
Do	75	6.5	4.25	0.0
3 per cent agar	Control.	6.5	6.50	Trace.
Do	5	6.5	6.40	46.4
Do	10	6.5	5.80	74.1
Do	15	6.5	5.30	88.2
Do	20	6.5	5.10	72.4
Do	25	6.5	4.95	39.6
Do	30	6.5	4.85	21.8
Do	50	6.5	4.55	Trace.
Do	75	6.5	4.30	0.0
10 per cent gelatin	Control.	6.7	6.70	Trace.
Do	5	6.7	6.30	39.2
Do	10	6.7	5.90	53.4
Do	15	6.7	5.65	68.8
Do	20	6.7	5.25	54.6
Do	25	6.7	5.10	43.2
Do	30	6.7	4.95	24.1
Do	50	6.7	4.65	Trace.
Do	75	6.7	4.50	0.0
Silicate gel	Control.			0.0
Do	5			4.4
Do	10			12.6
Do	15			28.2
Do	20			14.2
Do	25			3.8
Do	30			Trace.
Do	50			0.0
Do	75			0.0
Collodion	Control.			[a] Trace.
Do	5			[a] 8.4
Do	10			[a] 74.6
Do	15			[a] 93.2
Do	20			[a] 45.6
Do	25			[a] 9.6
Do	30			[a] Trace.
Do	50			[a] Trace.
Do	75			[a] 0.0

[a] After 48 hours.

The results of 10 trials, as summarized in Table 5, show the germination in different media in the presence of various percentages of carbon dioxide. Table 5 also shows the acidity of the media at the beginning of the experiments and at the end of the germination period. The highest percentage of germination in each medium occurred in an atmosphere containing 15 per cent of carbon dioxide. Atmospheres containing this amount of carbon dioxide changed the hydrogen-ion concentration of the tap water from 6.5 to 4.9; that of agar from 6.5 to 5.3, and that of gelatin from 6.7 to 5.6. Kendall (*9*) showed that under ordinary conditions the partial pressure of carbon dioxide in the air produces a hydrogen-ion concentration in pure water of 5.69

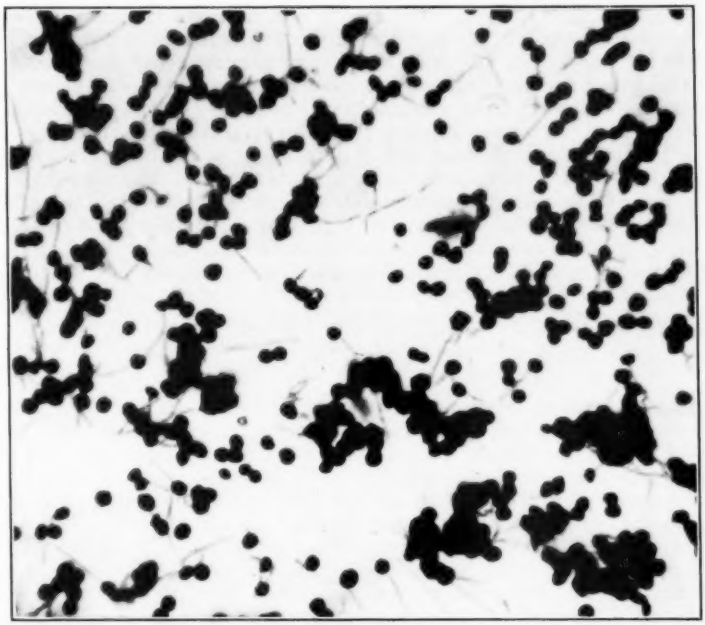

FIG. 3.—Germination of chlamydospores of *Ustilago zeae* on collodion in saturated atmosphere

and that atmospheres containing 100 per cent of carbon dioxide over water produce an acidity of P_H 3.9. Somewhat similar data are presented in Table 5, in which it is shown that atmospheres containing higher concentrations of carbon dioxide produced greater acidity of the media.

The optimum acidity for the germination of the spores, as obtained with carbon dioxide, is probably about P_H 4.9. This is the hydrogen-ion concentration produced in tap water subjected to atmospheres containing 15 per cent of carbon dioxide. Its optimum relation to the germination of the spores was observed in the water cultures and in the cultures in which the spores were dusted on collodion films or glass surfaces. In the use of collodion or glass surfaces as

substrata, the water of condensation was the only source of moisture and was sufficient for germination. In the case of the colloidal media (agar and gelatin), sufficient carbon dioxide to change the acidity of the entire mass to the optimum P_H changed the acidity of the surface film of water, the true medium of the germinating spores, to a point below the optimum. That this was the case was brought out in connection with the experiments in which bottles containing agar or gelatin were filled with mixtures of carbon dioxide and air, previously described. Furthermore, in atmospheres containing 15 per cent of carbon dioxide the surface film of water reached the optimum acidity long before an equilibrium was established throughout the entire medium.

It is interesting to note in Table 5 that excellent germination was obtained when silica jelly and collodion were used as substrata (fig. 3). Since De Bary (*1, p. 349–352*) made the statement that the nutrients in the media in which fungus spores are germinated play a part in their germination, that explanation has been almost universally accepted. The results obtained in the experiments presented in this paper by germinating the chlamydospores of *Ustilago zeae* on silica jelly or collodion do not confirm this theory. These media did not contain any nutrient material, and germination was therefore dependent upon temperature, moisture, carbon dioxide, and oxygen.

It appears from results obtained in these experiments that atmospheres containing 15 per cent of carbon dioxide produce in the culture media a hydrogen-ion concentration of 4.9, which is optimum to the germination of the chlamydospores of *Ustilago zeae*.

SUMMARY

Experiments on the stimulatory effect of the presence of living plant tissue upon the germination of the chlamydospores of *Ustilago zeae* (Beckm.) Ung. indicate that a great variety of plant tissues affect the germination of the spores.

Analysis of the stimulation caused by the presence of plant tissue shows that the stimulatory agent is carbon dioxide.

Atmospheres containing 15 per cent of carbon dioxide produced by the presence of plant tissue or by a gas generator were found to be optimum to the germination of the spores of *Ustilago zeae*.

A retarding effect upon the germination of these spores was obtained when atmospheres containing more than 15 per cent of carbon dioxide were used.

The stimulatory effect of carbon dioxide upon the germination of the spores of *Ustilago zeae* is apparently due to a definite action of carbonic acid.

Atmospheres containing 15 per cent of carbon dioxide produced hydrogen-ion concentrations in the spore culture media varying from 4.9 to 5.6, according to the medium used. The optimum acidity for the germination of the chlamydospores of *Ustilago zeae* obtained with carbonic acid is, however, probably nearer P_H 4.9 than P_H 5.6.

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NOTES ON THE LIFE HISTORY AND CONTROL OF THE STRAWBERRY LEAF ROLLER[1]

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INTRODUCTION

Life-history and control studies of the strawberry leaf roller (*Ancylis comptana* Fröhl.) were begun at Ames, Iowa, on June 15, 1923, and were carried on throughout the summer season. When the work was undertaken the larvae of the first generation were numerous, and some had pupated in the strawberry fields in the vicinity of Ames. By June 20 many moths were flying, and a great number of these were collected and placed on strawberry plants which had been potted and covered with 8-inch lantern-globe cages. The adults were kept confined in the cheesecloth-covered chimneys for oviposition records. Sweetened water, sprinkled on the leaves or on pieces of blotting paper, served as food.

LONGEVITY OF ADULTS

The average length of life of the 30 females under observation was found to be 14.73 days, the maximum 34 and the minimum 5 days. The average longevity of 25 males under observation was 16.56 days, the maximum 27 and the minimum 9 days. On June 27 a dozen moths were taken from a breeding cage and held in captivity without food. They lived from 5 to 16 days; on an average, 8.16 days. Detailed longevity data are given below.

Number of days adults lived	Males	Females	Number of days adults lived	Males	Females
5	----	1	17	2	----
8	----	4	18	5	2
9	1	1	19	2	1
10	2	1	21	1	3
11	1	2	22	1	2
12	1	5	24	1	----
13	3	----	25	1	----
14	3	1	27	1	----
15	----	2	34	----	1
16	----	4			

[1] Received for publication Aug. 23, 1926; issued February, 1927. Contribution from the Iowa Agricultural Experiment Station. Printed with the permission of the director.

[2] Formerly connected with the Iowa Agricultural Experiment Station.

OVIPOSITION

The preoviposition period ranged from 1 to 4 days, averaging 2.3 days. Fertilized females laid eggs quite freely while in captivity. Many newly emerged unfertilized females were placed in cages on strawberry plants, and at the end of the fifth day some had deposited infertile eggs and some showed no signs of oviposition. In all of the breeding work a newly emerged male and female were placed in a cage and kept together throughout the period of experimentation. In some cases, however, a male was used to fertilize a second female.

The oviposition period was determined by making daily egg counts and moving the pair of moths to a new cage containing an uninfested plant. The length of the oviposition period of 30 females follows. The oviposition period of the 30 females averaged 9.5 days, the minimum being 4 and the maximum 19 days. The post-oviposition period ranged from 1 to 21 days, the average being 3.3 days.

Number of females	Number of days in oviposition period	Number of females	Number of days in oviposition period
1	4	3	11
1	5	2	12
6	6	2	14
4	7	1	15
5	8	1	16
1	9	1	18
1	10	1	19

The number of eggs laid by the 30 females was as follows:

Number of females	Number of eggs laid	Number of females	Number of eggs laid
1	14	1	89
1	33	1	90
2	35	1	93
1	48	2	94
1	52	3	96
1	55	1	99
1	56	1	109
1	61	1	110
1	62	1	122
1	68	1	127
1	71	1	136
1	83	1	161
1	86	1	182

The number of eggs laid per day ranged from 1 to 67 and averaged 9.25. These moths deposited 78.26 per cent of their eggs during the first half of their lives. The average number of eggs laid by one individual was 85.1, the maximum was 182, and the minimum was 14.

INCUBATION PERIOD

The length of the incubation period of the strawberry leaf roller was determined from data taken from 919 hatchings under an outside shelter. Under these conditions when the temperature ranged from 50° to 100° F., the larvae issued 5 to 13 days after deposition, the average interval being 7.9 days (fig. 1). The following

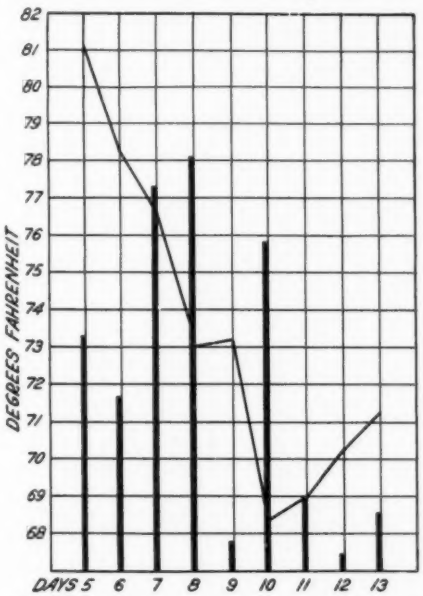

FIG. 1.—Relation of mean temperature to length of incubation period of the strawberry leaf roller. (The length of the bars corresponds to the number of individuals for which records were obtained, the distance from the base to the next horizontal line representing 20 individuals)

tabulation shows that 5 days is the length of the incubation period at 81.1° and that an incubation period of 8 days at 73.02° seems to be the optimum for development.

Number of individuals	Average temperature, °F.	Number of days in incubation period	Number of individuals	Average temperature, °F.	Number of days in incubation period
126	81. 1	5	176	68. 4	10
93	78. 2	6	41	69	11
206	76. 57	7	9	70. 3	12
222	73. 02	8	31	71. 3	13
15	73. 2	9			

HABITS OF LARVAE AND INJURY TO PLANTS

The little caterpillars usually feed on the dorsal surface of the leaf, but they may also be found feeding on the under side. The actual damage is caused by their feeding on the leaf tissue. The whole leaf is not eaten, but as a rule it is folded over at the midrib and held in this position by fine silken threads. In this fold the larva feeds on the epidermis, causing the rest of the tissues of this area to turn brown and die, thus making the strawberry foliage look as if it had been scorched by fire. Where the insects are abundant, the leaves, especially those near the bud, are often tied in an indiscriminate fashion.

LENGTH OF LARVAL PERIOD

Data showing the length of the larval period at Ames in 1923, are as follows:

The minimum period for larval development was 16 days at a mean temperature of 79.8°. The maximum period of the larval stages for

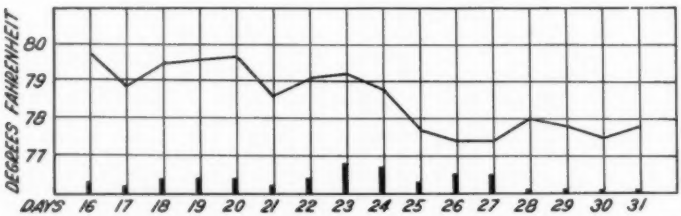

FIG. 2.—Relation of mean temperature to length of larval period of the strawberry leaf roller. (The length of the bars corresponds to the number of individuals for which records were obtained, the distance from the base to the next horizontal line representing 10 individuals)

the summer broods increased to 31 days with only a slight decrease in temperature (fig. 2). The average number of days required for development, based on all the records was 22.7.

Number of individuals	Average temperature, °F.	Number of days in larval stage	Number of individuals	Average temperature, °F.	Number of days in larval stage
3	79. 8	16	7	78. 8	24
2	78. 9	17	3	77. 7	25
4	79. 5	18	5	77. 4	26
4	79. 6	19	5	77. 4	27
4	79. 7	20	1	78	28
2	78. 6	21	1	77. 8	29
4	79. 1	22	1	77. 5	30
8	79. 2	23	1	77. 8	31

LENGTH OF PUPAL PERIOD

The length of the pupal period ranged from 6 to 15 days and averaged 9.6 days. Temperature seemed to have a direct bearing on the number of days required for development. Nevertheless, indi-

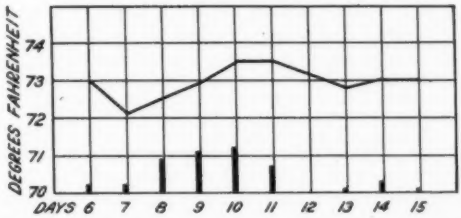

FIG. 3.—Relation of mean temperature to length of pupal period of the strawberry leaf roller. (The length of the bars corresponds to the number of individuals for which records were obtained, the distance from the base to the next horizontal line representing 10 individuals)

viduals that spent 6 and 15 days, respectively, in the pupal stage developed at a mean temperature of 73° F Other notable exceptions were individuals taking from 7 to 14 days to develop at temperatures ranging from 72.1° to 73.5°. The optimum for development seems to be 10 days at a mean temperature of 73.5° (fig. 3). Detailed records showing length of the pupal period at different temperatures are as follows:

Number of individuals	Average temperature, ° F.	Number of days in pupal stage	Number of individuals	Average temperature, ° F.	Number of days in pupal stage
2	73	6	7	73. 5	11
2	72. 1	7	1	72. 8	13
9	72. 5	8	3	73	14
11	72. 9	9	1	73	15
12	73. 5	10			

TOTAL LIFE CYCLE

Summing up the averages of the number of days required for the development of the different stages, Table 1, gives an average total life cycle of 42.5 days.

TABLE 1.—*Average length of life cycle*

	Number of records	Maximum	Minimum	Average
Number of days of incubation of eggs	919	13	5	7.9
Larval period (days)	55	31	16	22.7
Pupal period (days)	48	15	6	9.6
Preoviposition period (days)	30	4	1	2.3
Total number of days				42.5

SEASONAL HISTORY

In 1923 at Ames there were three complete generations of the strawberry leaf roller. Observations in Iowa from 1915 to 1918 definitely established the fact that the leaf roller spends the winter in the strawberry beds as a nearly full-grown caterpillar. Early in the spring when the food supply is available the caterpillars feed to some extent and soon transform to the pupal stage, and a few days later the adult moths appear. These moths of the overwintered brood lay eggs and give rise to the first generation, which appears in late May and early June.[3] By June 15, when this investigation was begun, larvae of all sizes and some pupae were found in the leaves.

By June 29, most of the larvae of this first generation had pupated, and many moths were flying in the fields. By July 3 there were scarcely any larvae and but few pupae of this group to be found, and on July 10 small larvae of the second generation were very numerous in the fields. On August 2 a few moths were emerging, and many full-grown larvae were observed, some of which were pupating. By the 10th of August the moths were emerging freely. The eggs which they deposited gave rise to the third generation. The larvae of this third generation, which fed actively until the strawberry leaves were no longer suitable for food, prepared for themselves quarters for hibernation in the strawberry foliage in the beds.

There was an overlapping of these generations, owing to the great variation in the length of the larval period and some variation in the length of the other developmental stages.

NATURAL ENEMIES

During the latter part of June many spiders were found in the strawberry fields, and most of them were on the rolled leaves. It is thought that they were feeding on the larvae of the leaf roller.

Another important factor in the natural control of these rollers is certain parasitic hymenopterous insects which frequently parasitize the larvae or pupae. These parasites were not found in great numbers, but certain species occasionally became sufficiently abundant to check rapid multiplication of the leaf rollers.

During the summer of 1923 at Ames the writer bred the following parasites from parasitized larvae of *Ancylis comptana: Sympiesis ancylae* Girault, *Meteorus trachynotus* Vier, *Hoplocryptus incertulus* (D. T.), *Spilochalcis albifrons* Walsh, and *Spilocryptus polychrosidis* Cush.[4] The pupal stage of these parasites lasted from five to nine days when they were placed in the cool part of the insectary. All specimens were reared in salve boxes on damp sand.

CONTROL EXPERIMENTS

When the rollers become very abundant control measures must be instituted if a full crop of strawberries is to be harvested. In spite of some natural enemies these insects often breed in great numbers and almost defoliate the plants.

[3] WEBSTER, R. L. THE STRAWBERRY LEAF ROLLER (ANCYLIS COMPTANA FRÖHL). Iowa Agr. Expt. Sta. Bul. 179 (abridged), 4 p., illus. 1918.

[4] The determinations were made by R. A. Cushman and A. B. Gahan, of the Bureau of Entomology, U. S. Department of Agriculture.

The spraying and dusting experiments described in this paper were conducted on an acre of strawberries made available by the horticultural department of Iowa State College. The spraying and dusting were begun June 27, 1923, just as the moths were beginning to oviposit for the second generation. Inasmuch as some of the poison was washed off by a heavy rain late in the afternoon, the spraying and dusting were repeated on July 5. The spray and dust were next applied on July 29, when the moths were beginning to oviposit for the third generation. These tests were made on moderately infested plants which had been set out in the fall of 1922. The rows were 3 feet apart in the field and ran north and south. The plants were spaced about 18 inches in the drill throughout the field. Along the east side of the field were three strawberry patches, one of which was several years old and badly infested. The second and third patches had been set out about two years before the time of this investigation and were less heavily infested. These small adjoining patches covering altogether about one-fourth of an acre, were used as far as possible as a control.

Since it is almost impossible to kill the larvae and pupae in the rolled leaves, the applications of arsenicals were timed to poison the young larvae as they issued from the eggs and started to feed. The following insecticides were used:

Dusts:

Lead arsenate, 1 pound to 5 pounds of gypsum.
Calcium arsenate, 1 pound to 10 pounds of gypsum.
Lead arsenate, 1 pound to 5 pounds of slaked lime.
Calcium arsenate, 1 pound to 10 pounds of slaked lime.

Sprays:

Lead arsenate, 1 pound to 50 gallons of water.
Lead arsenate, 1½ pounds to 50 gallons of water.
Lead arsenate, 2 pounds to 50 gallons of water.
Lead arsenate, 1 pound to 50 gallons of water, with 1½ pounds of laundry soap.
Lead arsenate, 1½ pounds to 50 gallons of water, with 1½ pounds of laundry soap.
Lead arsenate, 2 pounds to 50 gallons of water, with 1½ pounds of laundry soap.

The dusts were applied with a hand duster at the rate of about 25 pounds to the acre; the sprays were applied with a wheelbarrow sprayer at the rate of about 100 gallons to the acre.

After the first application no detailed counts were made to determine the exact effect of the poison on the larvae, but it was easy to see that the larvae were much less numerous on the treated plots than on the controls. On July 29, the second application was made, and on August 3 every plant in the field was thoroughly examined to determine the effectiveness of the different arsenicals. Table 2 shows the results obtained. From this table it is apparent that at the conclusion of the experiments the insects were under almost complete control in all the plots in spite of the fact that they are especially fond of new plants. Only 357 rollers, too few to cause any appreciable damage, were found in the entire acre. The most effective of the arsenicals used was lead arsenate applied at the rate of 1 pound to 5 pounds of gypsum. Lead arsenate at the rate of 1 pound to 5 pounds of slaked lime and calcium arsenate at the rate of 1 pound to 10 pounds of gypsum gave nearly as good results.

The dust consisting of 1 pound of calcium arsenate and 10 pounds of slaked lime was about as effective as the various sprays. Since such small numbers of rollers were dealt with it is almost impossible to interpret the data correctly, but it is seen that where the two sprays were applied which contained only 1 pound of the arsenical to 50 gallons of water the infestation was greater than where sprays were used that contained more of the insecticide.

TABLE 2.—*Comparative effectiveness of the different poisons used against the strawberry leaf roller at Ames, Iowa, in 1923*

Plot No. [a]	Poison used	Number of rollers found after arsenical was applied
1	Lead arsenate, 1 pound to 5 pounds of gypsum (dust)	6
2	Calcium arsenate, 1 pound to 10 pounds of gypsum (dust)	7
3	Lead arsenate, 1 pound to 5 pounds of slaked lime (dust)	7
4	Calcium arsenate, 1 pound to 10 pounds of slaked lime (dust)	42
5	Lead arsenate, 1 pound to 50 gallons of water (spray)	63
6	Lead arsenate, 1½ pounds to 50 gallons of water (spray)	45
7	Lead arsenate, 2 pounds to 50 gallons of water (spray)	46
8	Lead arsenate, 1 pound to 50 gallons of water and 1½ pounds of laundry soap (spray)	59
9	Lead arsenate, 1½ pounds to 50 gallons of water and 1½ pounds of laundry soap (spray)	46
10	Lead arsenate, 2 pounds to 50 gallons of water and 1½ pounds of laundry soap (spray)	36
	Total	357

[a] The area of each plot was one-tenth of an acre.

Three of the dusted areas showed considerably less infestation than the sprayed plots, in spite of the fact that plots 1, 2, and 3 were nearer the old strawberry beds than were the dusted areas. Plot 1 was nearer the old beds than was plot 2, and plot 2 was nearer than plot 3. Almost all the plants in the patches used as controls were completely defoliated when these counts were made.

A STUDY OF DARK, HARD KERNEL AND PROTEIN CONTENT OF HARD RED SPRING WHEAT[1]

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INTRODUCTION

Market grades for hard-wheat types have usually provided a classification on the basis of dark or hard-kernel content. For a number of years before the present Federal grades were promulgated the dark, hard-kernel content was used as a basis for grading spring wheat. The present Federal grades provide for three subclasses of hard red spring wheat, as follows: (1) Dark northern spring, containing 75 per cent or more of dark, hard, and vitreous kernels; (2) northern spring, containing less than 75 per cent and more than 25 per cent of dark, hard, and vitreous kernels; and (3) red spring, containing not more than 25 per cent of dark, hard, and vitreous kernels. Hard spring, containing 85 per cent or more of dark, hard, and vitreous kernels, is a premium grade under the subclass dark northern spring.

The present Federal grades provide similar subclasses for hard red winter and durum wheats. The use of the dark or hard-kernel content as a basis for grading has been confined to "hard" wheat types, which will generally average higher in protein or gluten content than other types, and which will also show greater variation in physical characteristics and composition according to season and location. Dark or hard-kernel content is used as a basis for grading hard wheats on the assumption that the percentage of dark, hard, vitreous kernels is an indication of the protein content of the wheat.

It is generally conceded that dark, hard, vitreous kernels contain more protein than light-colored, starchy kernels from the same lot of wheat. Snyder[2] found that "when the two types of seed, light and dark, were selected from the same lot of wheat, the darker seeds in all samples analyzed were found to be richer in protein." Snyder also found that light seeds and dark seeds from different sources contained different amounts of protein.

Roberts[3] attempted to correlate crushing strength of hard winter wheat with protein content, but found no significant correlation. The results of his work indicate that crushing or breaking strength is probably of less importance as indicating protein content than the color of the kernel.

Mangels and Sanderson[4] found a positive correlation between protein content and dark, hard kernels for the crops of 1922, 1923,

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[1] Received for publication Aug. 19, 1926; issued February, 1927. Contribution from the department of Milling; published with the permission of the director of experiment station.

[2] SNYDER, H. WHEAT AND FLOUR INVESTIGATIONS. Minn. Agr. Expt. Sta. Bul. 85: 179-224, illus. 1904.

[3] ROBERTS, H. F. RELATION OF HARDNESS AND OTHER FACTORS TO PROTEIN CONTENT OF WHEAT. Jour. Agr. Research 21: 507-522, illus. 1921.

[4] MANGELS, C. E., and SANDERSON, T. THE CORRELATION OF THE PROTEIN CONTENT OF HARD RED SPRING WHEAT WITH PHYSICAL CHARACTERISTICS AND BAKING QUALITY. Cereal Chem. 2: 107-112. 1925.

and 1924, but the coefficient of correlation showed considerable seasonal variation.

Under the Federal system of grading hard red spring and hard red winter wheat, the term "dark, hard, and vitreous" kernels is used, but in actual practice wheats are divided into subclasses on the basis of color only. Kernels which are dark in color, however, are usually hard and vitreous, and at present no convenient or practical method of measuring "hardness" or "vitreousness" is available.

The North Dakota Experiment Station has collected data on dark kernels and protein content for four crop years, and now has information on more than 1,000 samples from the crops of 1922 to 1925, inclusive. Because of the importance of the relationship between dark kernels and protein content it was deemed advisable to make a careful study of these data.

CORRELATION BETWEEN PROTEIN CONTENT AND PERCENTAGE OF DARK KERNELS IN HARD RED SPRING WHEAT

The results of correlation studies made on the North Dakota crops of 1922 to 1924 have already been published.[4] Table 1 brings together the results of correlation studies for the four crop years 1922 to 1925, inclusive.

TABLE 1.—*Correlation between protein content and dark-kernel content of hard red spring wheat from North Dakota crops, 1922–1925*

Year	Number of samples	Mean protein content	Mean dark-kernel content	Coefficient of correlation	Probable error
		Per cent	*Per cent*		
1922	90	12.12	83	0.660	±0.041
1923	199	13.35	73	.067	±.047
1924	316	11.35	79	.453	±.030
1925	436	12.31	90	.299	±.043

The coefficients of correlation for the four crop years show a wide range in magnitude. In the 1922 crop there is a marked correlation between dark kernels and protein content, and in the 1924 crop there is a significant correlation, but in the 1925 crop the correlation is less than 0.3, and in 1923 there is practically no correlation. The 1923 crop also shows the highest mean protein content and the lowest mean dark kernel content, but this apparent discrepancy is due to the fact that many of the samples from the 1923 crop were weather damaged. These weather-damaged samples while high in protein content would on analysis show a large percentage of light-colored or starchy kernels.

A study of frequency diagrams indicates that wheat having a high percentage of dark kernels will show considerable variation in protein content. The frequency diagram for the 1925 crop shows that wheat containing 95 to 100 per cent of dark kernels varied from 10 to 19 per cent in protein content. The coefficient of correlation of the 1922 crop is high because of the fact that samples from this crop containing a high percentage of dark kernels show relatively less spread in protein content than other crops.

[4] MANGELS, C. E., and SANDERSON, T. THE CORRELATION OF THE PROTEIN CONTENT OF HARD RED SPRING WHEAT WITH PHYSICAL CHARACTERISTICS AND BAKING QUALITY. Cereal Chem. 2: 107–112. 1925.

FREQUENCY DISTRIBUTIONS OF SAMPLES ON THE BASIS OF DARK-KERNEL CONTENT

A study of frequency distribution of samples on the basis of dark-kernel content has brought out some interesting facts.

TABLE 2.—*Frequency distribution of samples of hard red spring wheat on the basis of dark-kernel content*

Class[a]	1922 crop		1923 crop		1924 crop		1925 crop	
	Frequency	Percentage of total samples	Frequency	Percentage of total samples	Frequency	Percentage of total samples	Frequency	Percentage of total samples
10	0		2	1.00	12	3.79	0	
20	0		5	2.51	8	2.53	0	
30	0		7	3.51	6	1.89	2	0.45
40	2	2.22	13	6.53	6	1.89	5	1.14
50	3	3.33	14	7.03	12	3.79	12	2.75
60	8	8.88	20	10.05	13	4.11	21	4.81
70	10	11.11	25	12.56	31	9.81	21	4.81
80	16	17.77	38	19.09	53	16.77	47	10.77
90	29	32.22	50	25.12	109	34.49	77	17.66
100	22	24.44	25	12.56	66	20.88	251	57.56
Total	90		199		316		436	

[a] The class mark denotes the mid-point of the group. For example, the 80 class includes all samples containing from 75 to 85 per cent kernels, the last class (100) includes samples containing from 95 to 100 per cent of dark kernels.

Table 2 gives frequency distribution on the basis of dark-kernel content for samples from the four crops studies. Figures 1 and 2 show distribution for the crops of 1924 and 1925. It will be noted from Table 2 that in all the crop years recorded more than half the samples were found in the three highest classes; which means that when graded more than half of the total samples would fall into the dark northern spring subclass. The data in Table 2 were obtained through crop surveys and, in the writer's opinion, represent a fairly accurate cross section of the North Dakota crop for the years given. Table 2 indicates that in 1925 over 85 per cent of the North Dakota crop would fall in the dark northern spring subclass, that is, it contained 75 per cent or more of dark kernels.

The frequency distributions of samples on the basis of dark-kernel content when plotted give, with one exception, the same type of curve.[5] The frequency distribution curves from the crops of 1922, 1923, and 1924 can be shown by calculation to be Pearson's normal type I curve, and in all cases are skewed to the right. The data for 1925 give a Pearson's J-shaped type I curve. All the distribution curves show similarity in that they are skewed to the right.

FREQUENCY DISTRIBUTIONS OF SAMPLES ON THE BASIS OF PROTEIN CONTENT

The frequency distributions of samples on the basis of protein content are shown in Table 3 and in Figure 3. For three of the four years (1922, 1924, and 1925) the greatest frequency is found at 11 per cent. The frequency distribution curves for these data (fig. 3) can be shown by calculation to be Pearson's type I curve, that is,

[5] PEARSON, K. TABLES FOR STATISTICIANS AND BIOMETRICIANS. PART I. Ed. 2, 143 p., illus. [Cambridge, Eng. 1924.]

with the exception of the 1924 crop, in which the distribution apparently follows Pearson's type VI curve. The curves for these distributions show similarity in that they are in all cases skewed to the

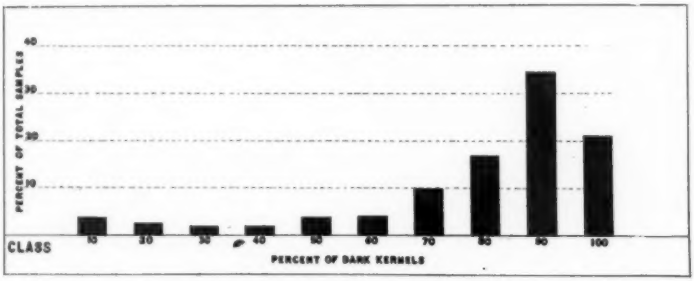

FIG. 1.—Distribution on the basis of dark-kernel content of samples from North Dakota spring-wheat crop for 1924

left. The 1923 data show the least and the 1925 the greatest skewness. The frequency distributions for protein content show appreciable skewing to the left, but as has been previously pointed out, the frequency distributions on the basis of dark-kernel content

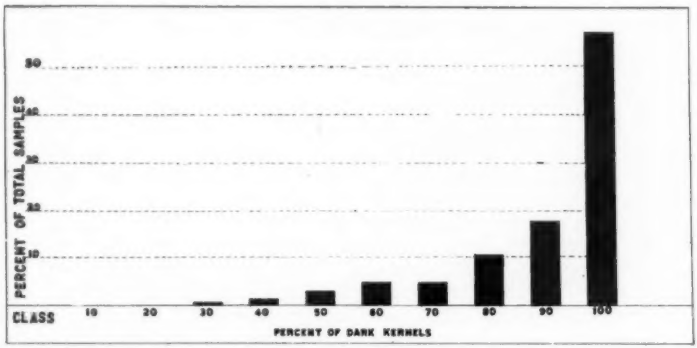

FIG. 2.—Distribution on the basis of dark-kernel content of samples from North Dakota spring-wheat crops, 1925

in all cases were skewed to the right. If a high degree of correlation existed between protein content and dark-kernel content, one would expect to find the distributions skewed in the same direction.

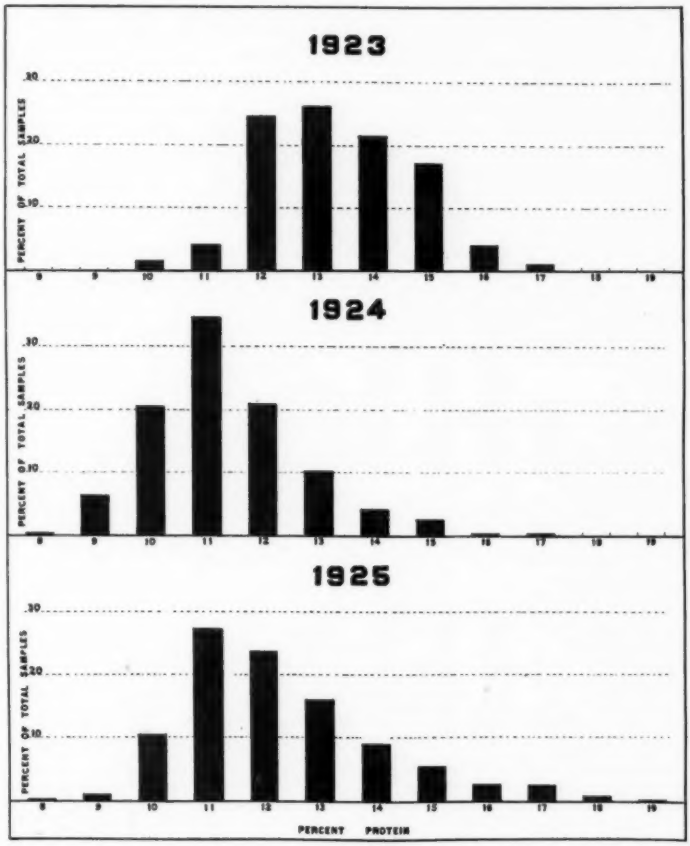

FIG. 3.—Distribution on the basis of protein content of samples from North Dakota spring-wheat crops, 1923–1925

TABLE 3.—*Frequency distribution of samples of hard red spring wheat on the basis of protein content*

Class [a]	1922 crop		1923 crop		1924 crop		1925 crop	
	Frequency	Percentage of total samples	Frequency	Percentage of total samples	Frequency	Percentage of total samples	Frequency	Percentage of total samples
8	0		0		1	0.32	1	0.23
9	3	3.33	0		20	6.33	4	.92
10	11	12.22	3	1.51	65	20.57	45	10.32
11	24	26.67	8	4.02	109	34.49	119	27.41
12	16	17.78	49	24.62	66	20.89	104	23.74
13	17	18.87	52	26.13	32	10.12	70	16.06
14	14	15.56	43	21.61	13	4.11	40	9.17
15	2	2.22	34	17.09	8	2.53	24	5.50
16	2	2.22	8	4.02	1	.32	12	2.75
17	1	1.11	2	1.01	1	.32	12	2.75
18	0		0		0		4	.92
19	0		0		0		1	.23
Totals	90		199		316		436	

[a] The class mark denotes the mid-point of the class. For example, the 12 per cent class includes all samples which fall within the range of 11.5 to 12.5 perent protein.

PROTEIN VARIATION AND DARK-KERNEL CONTENT

The wide variation in protein content of samples showing a high percentage of dark kernels was discussed briefly under correlation studies. Figures 4, 5, and 6 show the spread in protein content for (1) all samples, (2) samples containing more than 75 per cent of dark kernels, and (3) samples containing more than 85 per cent of dark kernels.

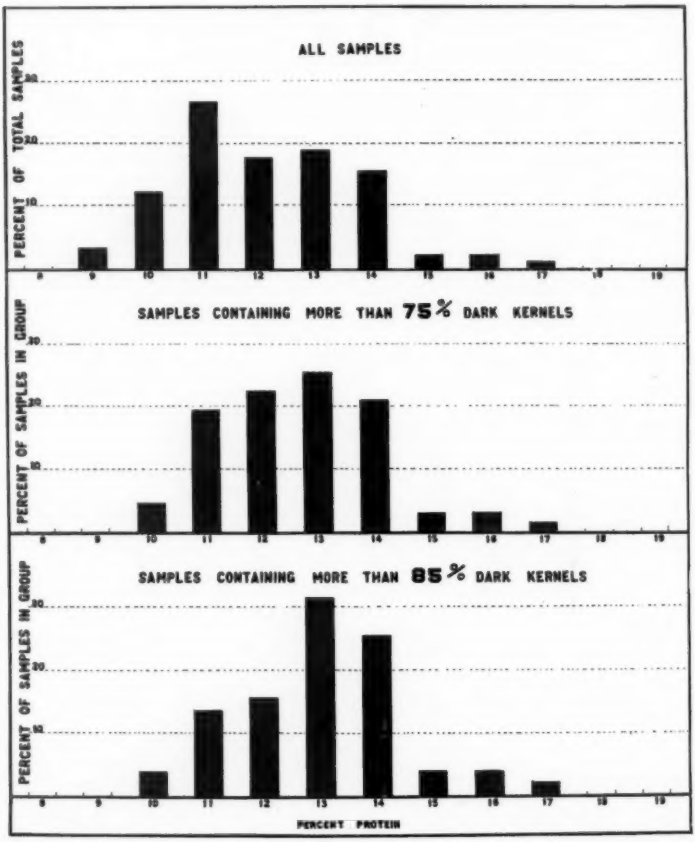
ALL SAMPLES
PERCENT OF TOTAL SAMPLES
SAMPLES CONTAINING MORE THAN 75% DARK KERNELS
PERCENT OF SAMPLES IN GROUP
SAMPLES CONTAINING MORE THAN 85% DARK KERNELS
PERCENT OF SAMPLES IN GROUP
PERCENT PROTEIN

FIG. 4.—Distribution on the basis of protein content of samples from North Dakota spring-wheat crops for 1922

Figures 4, 5, and 6 show that the spread in protein content in groups containing more than 75 per cent or more than 85 per cent of dark kernels is almost as large as the protein spread for all samples. In 1925 the protein content for all samples varied from 8 to 19 per cent, as shown by Figure 3, but the spread for the 75 and 85 per cent dark-kernel groups was almost as great—9 to 19 per cent. In 1924 the protein varied in all samples from 8 to 17 per cent, and the variation

for the 75 and 85 per cent dark-kernel groups was from 9 to 17 per cent. The 1923 crop averaged higher in protein than the 1924 and 1925 crops, but the spread in protein content for all samples was from 10 to 17 per cent, and the variation in the 75 and 85 per cent dark-kernel groups was from 11 to 17 per cent. The 1922 crop shows a greater degree of correlation between dark kernels and protein content

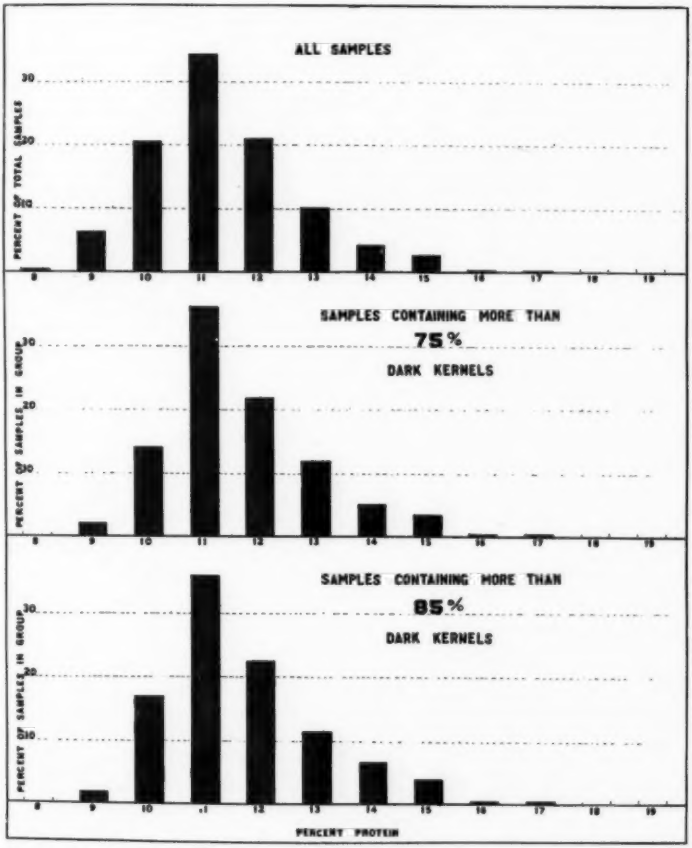

FIG. 5.—Distribution on the basis of protein content of samples from North Dakota spring-wheat crop for 1924

than other crops studied, but the spread in protein content for all samples of the 1922 crop was from 9 to 17 per cent, and the variation in the 75 and 85 per cent dark-kernel groups was from 10 to 17 per cent.

For the 1924 and 1925 crops the greatest frequency (figs. 5 and 6) for all samples is found in the 11 per cent class, but the greatest frequency for the 75 and 85 per cent groups is also found in the 11 per cent class.

For the 1923 crop the greatest frequency is found in the 13 per cent class for all samples, but the greatest frequency for the 75 per cent dark-kernel group again occurs in the same class (13 per cent).

The 1922 crop (fig. 4), as would be expected from the correlation coefficient, differs quite distinctly from the other crops. The greatest frequency for all samples of the 1922 crop occurs in the 11 per cent

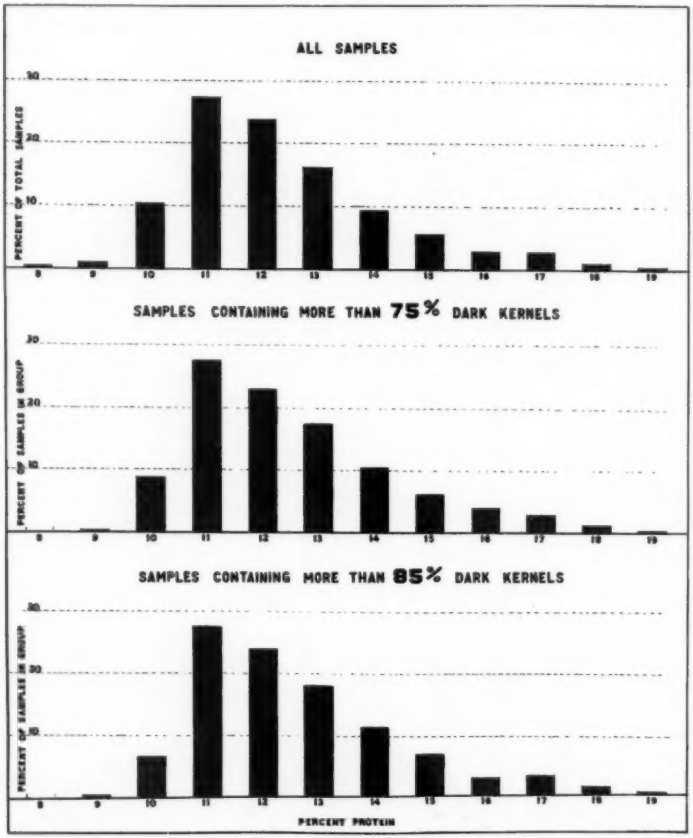
ALL SAMPLES
PERCENT OF TOTAL SAMPLES
SAMPLES CONTAINING MORE THAN 75% DARK KERNELS
PERCENT OF SAMPLES IN GROUP
SAMPLES CONTAINING MORE THAN 85% DARK KERNELS
PERCENT OF SAMPLES IN GROUP
PERCENT PROTEIN

FIG. 6.—Distribution on the basis of protein content of samples from North Dakota spring-wheat crop for 1925

class, and in this respect the crop is similar to the 1924 and 1925 crops, but the greatest frequency for the 75 per cent and 85 per cent dark-kernel groups is found in the 13 per cent class.

In Table 4 the samples are grouped in such a way as to bring out the relation of dark-kernel protein content to protein content of all samples. As will be noted, the 1922 crop shows a larger increase in protein for the 75 and 85 per cent dark-kernel groups than any crop of the other three years.

TABLE 4.—*Comparison of mean protein content of dark kernels and of kernels of all samples in hard red spring wheat, 1922–1925*

Year	Mean protein content of—		
	All samples	Samples containing more than 75 per cent of dark kernels	Samples containing more than 85 per cent of dark kernels
	Per cent	*Per cent*	*Per cent*
1922	12.12	12.67	12.98
1923	13.35	13.48	13.45
1924	11.35	11.56	11.66
1925	12.31	12.43	12.53

In the 1922 crop there is apparent a significant degree of correlation between dark kernels and protein content, but it was in 1922 that the protein test was first used extensively on the Minneapolis market. The 1922 crop shows a higher degree of correlation than the crops which followed, but even with this crop, the dark-kernel content was not considered sufficiently accurate as an index of protein content, and the grain trade began to use the protein test. The dark-kernel content is apparently of very questionable value in estimating the potential baking value of a lot of wheat. A high percentage of dark kernels does not assure the wheat buyer that he is getting a wheat that is high in protein, and for this reason the protein test is now considered by many buyers of equal importance with the grade certificate.

SUMMARY

Correlation between dark kernels and protein content shows considerable seasonal variation, and the degree of correlation may be quite low.

Frequency distributions of representative samples from North Dakota spring-wheat crops indicate that in every year of the four recorded a large proportion of the crop contained a high percentage of dark kernels and would fall in the highest subclasses when graded.

Frequency distributions of samples on the basis of dark-kernel content are skewed to the right, while distributions on the basis of protein content are skewed to the left, a fact which indicates a lack of close relationship between dark kernels and protein content.

Frequency distributions of samples containing more than 75 per cent or more than 85 per cent of dark kernels, respectively, when compared to distributions for all samples show (1) that total spread or variation in protein content is almost as great in 75 per cent or 85 per cent dark-kernel groups as in the total samples; (2) when distributions of all samples are compared with distributions of samples containing more than 75 per cent or more than 85 per cent of dark kernels, the greatest frequency falls in the same class in all groups, except in the case of the 1922 crop, where the greatest frequency moves up two classes—that is, into the 75 and 85 per cent

dark-kernel groups; (3) the mean of samples containing 85 per cent and 75 per cent, respectively, of dark kernels is very little larger than the mean of all samples except in 1922, when a significant increase is noted.

The color of the kernel is not a sufficiently accurate index of protein content to be used by the wheat buyer, and for this reason is being superseded by actual protein test. As an index of wheat quality, dark-kernel content is of questionable value.

THE COMPUTED AS COMPARED WITH THE DIRECTLY OBSERVED FASTING KATABOLISM OF CATTLE AS A MEASURE OF THE MAINTENANCE REQUIREMENT OF ENERGY [1]

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INTRODUCTION

The maintenance requirement of energy is the amount that is necessary to keep an animal in energy balance, that is, without energy gain or loss, under the standard conditions of activity to which the measure is to apply. This maintenance requirement is, therefore, a charge or expense which must be met before there can be a profitable product from feeding, and it is the most definite and practical fixed value in terms of which to reckon a rate of feed supply, that is, a plane of nutrition. It is also of special significance in the work of the Institute of Animal Nutrition of Pennsylvania State College, inasmuch as the main program of research, from the beginning, has been the determination of net energy values of feeds and net energy requirements of animals, in the ascertaining of both of which measurements separate estimations of the maintenance requirement of energy must be made. In view, further, of various special technical difficulties involved in determining the maintenance requirement, the principles and methods involved have been at all times matters of critical concern; and since there is occasion at this time to change the practice of the institute in determining the maintenance requirement of cattle it becomes desirable in this relation to review the development of the ideas held and methods followed by Armsby from the beginning of his respiration-calorimetric work at this institute in 1901 until his death in 1921.

DISCUSSION OF WORK OF ARMSBY AND OTHERS

The standard measure of the maintenance requirement of energy, with all species of animals, is the heat production during fast—a definition unequivocally accepted and at all times followed by Armsby, and discussed at length in United States Bureau of Animal Industry Bulletin 143 (*5, p. 41*).[2]

It is also a noteworthy fact that Armsby's standard for maintenance requirement of energy of swine, recommended by him in 1917 (*7*), is based entirely on measurements of the katabolism of fasting animals.

In the case of ruminants, the question has been not as to the standard but as to the method of measurement—as to whether the

[1] Received for publication Oct. 27, 1926; issued February, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 178.

fasting katabolism is to be determined directly, as the heat production of a fasting animal, or, in view of the alleged impracticability of the direct determination, whether it is to be computed from the difference in heat production and difference in feed between two periods differing as to feed intake.

Because of the great capacity and the complicated anatomy of the ruminant alimentary tract and the slow and indirect course of the food and its residues in digestion, Armsby assumed at the beginning of his work in animal calorimetry that in the measurement of heat production during fast the continued presence of feed residues in the alimentary tract would affect the heat production, presumably either through fermentation or through the utilization of nutrients, and therefore that it is impracticable to determine directly the fasting katabolism of cattle.

The plausibility of this assumption is indicated by the conclusions of Ewing and Smith (*13*), who computed from the feed intake, the rate of fecal outgo, and the contents of the alimentary tract of steers, as determined at the slaughter of the animals after they had been fed rations composed of different proportions of cottonseed meal and corn silage, that the rate of passage of the residues through the animals varied from 2.92 days to 5.24 days.

This fact, which is generally appreciated as a result of observations of various kinds, is naturally discouraging to the idea of getting cattle into a status of complete fast.

The writers will show, however, that these difficulties are readily surmountable; that it is entirely practicable to get cattle into a status of essentially complete fast; and that the direct measurement of the heat production of cattle during fast affords a measure of the maintenance requirement of energy which is more satisfactory on several accounts, but especially because it is much less variable, than is a computed value. In the development of this point of view and with the same understanding of the problem, the writers are concluding a study begun by Armsby, in the last experiment conducted under his direction in 1920–21.

In the light of their earlier understanding of this situation, Armsby and Fries wrote, in 1903 (*8*), in the report of their first year's calorimetric experimentation on the available energy of timothy hay (experiment 174), that it was impracticable to determine directly the fasting katabolism—a view which Armsby expressed again and again in later publications. The following statement by Armsby (*7, p. 285, 288, 290*) in 1917, expresses very clearly a late view which he held on this matter:

> With animals such as man, carnivora, or swine, having a comparatively simple digestive apparatus and consuming relatively concentrated feed, the fasting energy expenditure can be determined without special difficulty by depriving the resting animal of feed during a relatively short period and measuring the katabolism with the aid of a respiration apparatus or calorimeter. The total amount of heat produced, determined either directly or by calculation, furnishes the measure of the energy expenditure and therefore of the net energy requirement for maintenance. * * *
>
> In the case of ruminants it is hardly practicable to determine directly the net energy requirement by measuring the katabolism of the fasting animal. Prolonged fasting would be required to free the voluminous and complicated digestive organs of these animals from feed residues, if this could be accomplished at all, and it would be difficult to determine when that point was reached, while it is questionable whether the results on such an animal could be regarded as normal

The fasting katabolism of such an animal may, however, be computed in the manner already described (374) from a comparison of two periods on different amounts of the same feed, or ration, both being less than that necessary for maintenance.

In reiterating the last statement later (*12, p. 44*) and in the actual use of the indirect method of obtaining the fasting katabolism, Armsby's view with regard to this matter was modified by the omission of the previously prescribed condition that the rations compared be less than that required for maintenance. This omission signifies the acceptance of the view that the utilization of feed energy is the same above and below the maintenance level.

The writers recently discussed the possibility that the directly observed heat production during fast might not be "normal," (*18*) at such length as seems justified by the slight evidence available on the subject, and concluded that even if the heat production during fast should include a factor of heat production of "abnormal" origin, characteristic of fasting, this fact would be without effect on the significance of the heat production of fast in relation to the energy required for maintenance, since the heat production of fast is the *measure* of the maintenance requirement of energy and of the net energy value of the amount of feed necessary to keep the animal in energy equilibrium.

The following example is presented as an illustration of the simplest use of the computation method of determining the fasting katabolism or maintenance requirement.

Experimental periods	Live weight	Dry matter of feed eaten	Heat production	Heat increment per kg. dry matter	Maintenance requirement of net energy
	Kgm.	*Kgm.*	*Cals.*	*Cals.*	*Cals.*
1	415.2	3.6674	8,224	706	5,635
2	429.7	5.4676	9,495		
Difference		1.8002	1,271		

The heat increment, or energy cost of feed utilization, is comprised of the aggregate expenditure of energy in prehension, mastication, deglutition, fermentation, rumination, peristalsis, digestion, transportation, dynamic stimulation, and excretion. It is computed by dividing the difference in heat production in the two periods by the difference in feed and multiplying the result by the dry matter of the feed. Then the maintenance requirement is computed by subtracting this total heat increment from the heat production. Thus, in this procedure, the heat production is computed to a basis of zero feed intake.

In the use of heat increments in determining the net energy values of feeds and the net energy requirements of animals a number of heat increments were usually computed from the several periods of an experimental program and used either individually or as an average (according to which of three methods was employed).

Two important weaknesses of the heat-increment method arise from the fact that each increment involves two experimental periods. Thus the resultant of the errors of heat measurement might be either

additive or compensating, and this distortion of values might be greatly magnified through their being referred to markedly different amounts of feed in the computation of the heat increments per unit of the ration.

These conditions have led to a most embarrassing diversity of heat-increment values, often derived from work which was really very good, and have required either a difficultly defensible averaging of discordant heat increments, or a more difficultly justifiable exclusion from the average of those heat increments which were most widely divergent from the average. In this situation it is obvious that the only other condition necessary to hopeless entanglement and confusion was the lapse of sufficient time to develop the effects of these inconsistencies.

A further difficulty in the heat-increment method of computation of the maintenance requirement seems to result from the assumption, which is fundamental to its use, that it varies directly as the dry matter of the feed. On account of the great variability of computed maintenance values and the fact that the computed maintenance from supermaintenance periods is always a materially lower value than is the directly determined fasting katabolism (compare Tables 1 and 2), the writers believe that the heat increment does not thus vary directly as the feed.

In order to appreciate the significance, consequences, and bearings of the facts as to the relation of the heat increment to the plane of nutrition, it is necessary to understand that the net useful energy of the ration, for any purpose, is the metabolizable energy (gross energy minus the energy of urine, feces, and methane) minus the heat increment.

The same principles, therefore, determine the utilization of the ration for maintenance as for production, and any use of heat increments determined at one level of feed intake in relation to the utilization of feed at another intake (as in the above numerical illustration) or the use of any single net energy value of a feed as applying to both maintenance and production, involves the assumption that the heat increment varies directly as the dry matter; that is, that it is a linear function of the amount of the feed.

In tracing the development and changes of Armsby's ideas as to the utilization of energy for maintenance, therefore, his observations on the utilization of energy for production will be considered as similarly relevant, since the heat increment is of like fundamental significance in both relations.

The following excerpts from and notes on the papers of Armsby and of Armsby and Fries are presented in chronological order. To avoid confusion, it is necessary to understand that Armsby used in his early writings the expression "net available energy," or sometimes simply "available energy," to mean net energy used for maintenance only, and spoke of the "utilization" of energy as meaning the use of energy over and above that required for maintenance for the production of body increase.

In 1903 Armsby and Fries (*8, p. 58, 64*) found the utilization ("availability") of metabolizable energy (feed minus excreta) to be 62.92 per cent on a plane of nutrition below maintenance, and 33.31 per cent for the production of body increase. They assumed that the net energy for maintenance and for production are *different linear*

functions of the metabolizable energy. In the following paragraph this conclusion is given in the author's own words:

> Our results indicate that the proportion of the metabolizable energy of the food which was utilized, above the maintenance requirement, to produce gain was decidedly less than that used below the maintenance requirement to prevent loss of tissue. In other words, they indicate that the conversion of digested and assimilated matter into actual tissue (fat) requires a considerable expenditure of energy, amounting in this case to about 47 per cent of the available energy or 30 per cent of the metabolizable energy. This result is quite in accordance with what we should anticipate. The digested matter of the food of herbivora appears to be resorbed chiefly in the form of carbohydrates and of organic acids. It seems altogether probable that a much less profound change is required to convert these resorbed products into forms suited to maintain the energy metabolism of the organism than is needed to convert them into the form of permanent tissue, especially fat.

In another 1903 publication Armsby (*1, p. 430*) similarly discussed the influence of amount of food on availability.

In 1905 Armsby and Fries (*9, p. 7, 42–44, 47*) discussed the different rates of use of energy for maintenance and for production and called attention to the harmony between this conclusion and the generally accepted principles of the physiology of nutrition. These ideas were also restated in three other publications during the next two years (*4, p. 281, 282; 2, p. 11–12; 3, p. 15*).

In 1911 Armsby and Fries wrote (*10, p. 48–49*):

> In previous publications we have expressed the belief, based in part upon experimental results and in part upon theoretical considerations, that this is not the case [that the availability of energy is the same above and below the maintenance requirement][3] but that the proportion of metabolizable energy capable of being stored up as gain after the maintenance requirement is satisfied is materially less than that available below the maintenance requirement to diminish the loss of energy from the body. The results of the present series of expriments fail to support that view.

After citing conflicting evidence and discrepancies (which can not conveniently be quoted), in accounts of both this and the earlier experiments, Armsby and Fries said:

> We are inclined to conclude that the percentage of the metabolizable energy of the grain available was substantially the same above and below the maintenance requirement and that the discrepancies in the earlier experiments are perhaps due to errors.

This was the beginning of Armsby's abandonment of the idea that the heat increment represented different linear functions of the metabolizable energy above and below maintenance. In the same bulletin Armsby and Fries (*10, p. 59*) suggested that hay, as distinguished from grain, may be more efficiently utilized below than above the plane of maintenance.

Conclusions on this subject during the remainder of Armsby's life expressed the same uncertainty that characterized the paper written in 1911. This situation obviously resulted from numerous imperfections of methods, as revealed by recent studies (*14, 15, 16, 20, 21, 23*), but especially from an imperfect method of computing the heat production to the standard day as to standing and lying.

Further expressions of Armsby's changed conception as to the relation of the heat increment to the plane of nutrition are commented upon below.

[3] Explanation in brackets is supplied by the authors of this paper.

In 1912 Armsby (*5, p. 9, 15, 36, 41, 42*) expressed continued adherence to the fasting katabolism as a measure of the maintenance requirement of energy, commented on its constancy as being in harmony with ideas expressed as to its origin and significance, and reaffirmed his belief that a larger percentage of the energy of hay is available below the point of maintenance than is utilized for gain above it.

In the bulletin on the maintenance ration of farm animals published in 1912, Armsby first described and used the heat-increment method of computing the maintenance requirement of net energy. In this relation he said:

> The experiments just cited are the only ones thus far reported in which this precise method of determining the maintenance requirement in terms of available energy has been followed.

In 1915 Armsby and Fries (*11, p. 476*) said:

> The data * * * fail to give any distinct evidence of a greater relative increase of heat production on heavy as compared with light feed or on supermaintenance as compared with submaintenance rations, the average tending, if anything, to be a trifle lower on the heavier rations.

Then, on account of the wide variability of the heat increments derived from the several periods of an experimental program, they expressed confidence in the increment derived from the greatest difference in feed, as minimizing the errors incident to the determination.

In 1916 Armsby (*6, p. 10*) reported net energy values primarily for maintenance or fattening, but said:

> There seems good reason for believing, however, that they may be taken without serious error to represent also the net energy values for growth and at least the relative values for milk production. Kellner believes that the same net energy values may also be regarded as expressing with sufficient accuracy the relative values of feeding stuffs for horses and for swine.

This expression seems to mark the completion of the change, begun in 1911, from the idea of the heat increment as representing different linear functions of the metabolizable energy for maintenance and production to one of the heat increment as a single straight-line function of the same.

In 1917 Armsby (*7, p. 279, 361, 450*) expressed the idea that net energy values for maintenance and production "might very well differ."

Explaining the theoretical ground for his original conception of net energy values for fattening being lower than for maintenance, he continued:

> Such data as are available, however, do not appear to indicate that this difference is a considerable one in the case of farm animals, and it would appear that, in the case of cattle at least and presumably in that of other species, the net energy values of feeding stuffs may be regarded as being substantially the same for fattening as for maintenance.

Further on, he said:

> There appears to be a somewhat general impression, however, that in addition to this effect on digestibility, the matter and energy actually resorbed from the ration become less efficient in producing gain as the amount of the ration is increased—in other words, that when the organism is flooded with the resorbed products of digestion, the katabolic processes are stimulated and a larger share of the energy of the digested matter escapes as heat. As appears in Chapter

XVII (764), the evidence on this point as yet seems hardly sufficient to warrant positive statements. The net energy values of feeding stuffs which have thus far been reported have been obtained chiefly in experiments on rations ranging from submaintenance to only moderately heavy fattening rations, and the results show no distinct indication of a decrease with increasing amounts of feed. On the other hand, physiological considerations render it quite conceivable that the effect of the feed in stimulating metabolism and so increasing the heat production (365) may be relatively greater on a high than on a low nutritive plane.

In 1918 Armsby, Fries, and Braman (*12, p. 44*) said, referring to the heat-increment method of computing the maintenance requirement:

> Strictly speaking, the foregoing method of computation assumes that the heat production caused by the feed is a linear function of its amount. This can not be regarded as having been proved, but no distinct indications to the contrary have appeared within the range of our experiments.

The article appearing in 1918 was Armsby's last published expression on this subject.

In Armsby's laboratory outline for experiment 221 E, the last research conducted under his direction, in 1920–21, occurred the following:

> Incidentally the basal katabolism of the animals as computed by a comparison of two periods on different amounts of feed is to be compared with katabolism as measured directly by the heat production after a longer or shorter period of fasting in order to ascertain whether it is possible to determine the basal katabolism of cattle by a single determination of the heat production in what corresponds to the "post-resorptive" state in man.

In regard, then, to the fundamental principle involved in the subject of this paper, namely, the relationship of the energy cost of feed utilization—the heat increment—to the amount of the feed, Armsby expressed himself in the publications of this institute during the years 1903 to 1907, inclusive, as in favor of the idea that the heat increment varied directly as the metabolizable energy of the ration, but at different rates for maintenance and production.

Beginning with the year 1911, he tended toward the belief in a single heat-increment value for the whole range of variation in plane of nutrition, first for grain alone, and later for hay and grain together; he used an original method for computing the maintenance requirement in a way to involve the assumption of a single heat-increment value; but he repeatedly called attention to the theoretical ground for belief in higher utilization (lower heat increments) for maintenance than for production, though in his last paper he said, in effect, that the idea of a single heat-increment value applying to production and to maintenance had not been proved; and in the outline for his last calorimetric experiment he showed by his plan to compare the directly determined with the computed maintenance requirement (a difference between which would indicate different rates of utilization of energy at different planes of nutrition) that he regarded this heat-increment-maintenance problem as still lacking final solution.

The computation method of determining the maintenance requirement of energy was used in all of the reports of respiration calorimetric studies at this institute until December, 1925, when, as a result of recent studies, a change to the directly determined value was announced by Forbes.[3] Notice of this change was also published

[3] FORBES, E. B. ADVANCES IN RESPIRATION CALORIMETRY WITH CATTLE. Amer. Soc. Anim. Prod. Proc. 1925–26. (In press.)

published in Science (*17*), and the data in three later papers (*17, 18, 19*) have been handled in the light of the new procedure.

The fact that the computed maintenance is significantly different from the directly determined value shows that energy is utilized for maintenance and for production at different rates of efficiency and, therefore, that the energy cost of feed utilization is not directly proportional to—that is, is not a linear function of—the dry matter or the metabolizable energy of the feed. The question as to whether the efficiency of utilization of energy for maintenance and for production can be represented as different linear functions of the dry matter or the metabolizable energy of the feed, as Armsby originally thought, or whether it is a curve, is not certain, and is being studied at this institute.

DATA SUPPORTING CHANGE IN METHOD

The following are the data on the strength of which the computation method has been abandoned in favor of the direct method of determining the fasting katabolism as a measure of the maintenance requirement of energy. The comparison of the computed and the directly measured katabolism discloses their respective degrees of variability and indicates the extent of their self-consistency and reliability.

Since the direct method has been adopted in principle only and has not been finally standardized, an arbitrary basis of comparison with the computation method was determined upon as the fairest practicable means of comparing the inherent variability of results obtained by the two methods.

Thus, in representing the direct method, the average heat production of each two consecutive days of fast was considered as an estimation of the maintenance requirement of energy, and for the indirect or computation method, the maintenance value derivable from each possible comparison of the heat production of two 2-day calorimeter periods was considered as a determination. On this basis 18 direct and 62 indirect determinations are compared.

In the actual use of heat increments in the computation method, as many determinations of the maintenance requirement are averaged as there are experimental periods; and in the use of the direct method the procedure will be standardized (as it has not yet been) in such a way that the heat production will be as nearly as possible that of a truly fasting condition.

The subjects in the last two experiments recorded in Table 1 (experiments 221D and 221F) were cows; in all the other experiments summarized in Table 1 the subjects were steers. In experiments 221D, 221E, and 221F of Table 2 the subjects were cows; in experiments 235 and 237 the subjects were steers.

TABLE 1.—*Computed fasting katabolism per 100 kilograms of live weight and per square meter of body surface*

Experiment No.	Animal	Periods compared	Average live weight	Computed fasting katabolism		
				Per head	Per 100 kgm. live weight	Per square meter body surface
			Kgm.	*Cals.*	*Cals.*	*Cals.*
174	I (steer)	A and B	393	6,206	1,579	1,335
		B and C	408	7,806	1,913	1,643
		C and D	419	6,581	1,571	1,360
		C and A	400	6,771	1,693	1,441
		D and A	404	6,740	1,668	1,425
		D and B	412	7,360	1,786	1,540
179	I (steer)	1 and 2	533	6,617	1,241	1,177
186	I (steer)	2a and 3a	571	7,509	1,315	1,279
		2b and 3b	571	8,641	1,513	1,472
190	A (steer)	3 and 4	275	4,546	1,653	1,222
200	A (steer)	3 and 4	403	6,222	1,544	1,318
207	A (steer)	3 and 4	511	5,113	1,001	935
190	B (steer)	3 and 4	192	4,494	2,341	1,513
200	B (steer)	3 and 4	303	5,828	1,923	1,475
207	B (steer)	3 and 4	380	5,552	1,461	1,220
208	C (steer)	2 and 3	264	3,576	1,355	988
		4 and 5	286	3,706	1,296	973
		5 and 6	280	3,415	1,220	908
		6 and 4	282	3,540	1,255	937
208	D (steer)	1 and 2	167	2,549	1,526	937
208	E (steer)	1 and 2	208	3,756	1,806	1,204
		2 and 3	201	3,658	1,820	1,195
		3 and 1	203	3,704	1,825	1,207
		5 and 6	206	3,070	1,490	990
209	F (steer)	1 and 2	297	4,923	1,658	1,262
		2 and 3	288	4,872	1,692	1,275
		3 and 1	292	4,891	1,675	1,267
		5 and 6	300	5,027	1,676	1,282
211	D (steer)	2 and 3	451	6,067	1,345	1,199
		1 and 4	458	6,188	1,351	1,211
		4 and 5	442	6,678	1,511	1,336
		5 and 1	444	6,528	1,470	1,303
211	G (steer)	2 and 3	378	5,665	1,499	1,251
		1 and 4	388	6,108	1,574	1,325
		4 and 5	376	4,848	1,289	1,073
		5 and 1	377	5,269	1,398	1,163
212	H (steer)	1 and 3	352	4,628	1,315	1,066
		3 and 5	346	4,097	1,184	955
		5 and 1	343	4,210	1,227	986
		2 and 4	349	4,716	1,351	1,094
		4 and 6	339	2,807	828	662
		6 and 2	339	3,208	946	757
216	J (steer)	1 and 2	378	5,144	1,361	1,136
		2 and 3	377	6,393	1,696	1,411
		3 and 4	372	5,477	1,472	1,220
		3 and 1	388	3,855	994	836
		4 and 1	372	5,055	1,359	1,126
		4 and 2	361	4,953	1,372	1,126
		5 and 6	404	4,483	1,110	948
		6 and 7	390	4,796	1,230	1,038
		7 and 5	390	4,726	1,212	1,023
217	J (steer)	1 and 2	513	5,574	1,087	1,015
220	K (steer)	1 and 2	505	6,670	1,321	1,228
		3 and 4	502	6,390	1,273	1,181
		4 and 5	502	7,397	1,474	1,367
		5 and 3	490	8,253	1,684	1,548
235	259 (steer)	3 and 2	365	4,499	1,233	1,013
235	260 (steer)	3 and 2	384	4,636	1,207	1,012
221D	886 (cow)	1 and 2	412	3,936	955	823
		3 and 2	422	3,873	918	797
221F	874 (cow)	1 and 2	423	5,298	1,252	1,090
221F	887 (cow)	1 and 2	328	4,360	1,329	1,051

Coefficient of variation of computed fasting katabolism per square meter of body surface, 17.93± 1.12 per cent; per 100 kilograms live weight, 19.52± 1.23 per cent.

TABLE 2.—*Observed fasting katabolism per 100 kilograms of live weight and per square meter of body surface*

Experiment and animal No.	Days of fast	Live weight	Heat production per standard day			Average heat production of 2 consecutive days	
			Per head	Per 100 kgm. live weight	Per square meter body surface	Per 100 kgm. live weight	Per square meter body surface
Experiment 235:		*Kgm.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Steer 260	Fourth	356	6,322	1,776	1,440	1,796	1,456
	5½ to 6½	354	6,430	1,816	1,471	1,827	1,479
	Seventh	351	6,453	1,838	1,487	1,805	1,459
	8½ to 9½	349	6,180	1,771	1,431		
Experiment 237:							
Steer 254	Third	339	6,517	1,922	1,555	1,908	1,536
	Fourth	334	6,324	1,893	1,516	1,882	1,498
	Fifth	328	6,137	1,871	1,479	1,847	1,452
	Sixth	323	5,886	1,822	1,425		
	Eighth	359	6,614	1,842	1,463	1,802	1,429
	Ninth	357	6,290	1,761	1,395	1,731	1,368
	Tenth	354	6,022	1,702	1,341		
Steer 36	Third	341	6,466	1,896	1,558	1,834	1,496
	Fourth	334	5,920	1,772	1,433	1,778	1,426
	Fifth	327	5,831	1,783	1,419	1,771	1,396
	Sixth	320	5,626	1,758	1,372		
Steer 47	Fourth	347	6,664	1,920	1,564	1,896	1,537
	Fifth	342	6,403	1,872	1,510	1,889	1,514
	Sixth	336	6,403	1,906	1,517		
Experiment 221 D:							
Cow 886	Second	411	6,779	1,649	1,404	1,615	1,368
	Third	405	6,400	1,580	1,331		
Cow 885	Second	420	6,523	1,553	1,339	1,541	1,326
	Third	417	6,374	1,529	1,312		
Experiment 221 E:							
Cow 885	Fifth	421	6,384	1,516	1,308	1,524	1,313
	Sixth	419	6,413	1,531	1,317		
Experiment 221 F:							
Cow 874	Eighth	395	6,906	1,748	1,469	1,723	1,446
	Ninth	393	6,669	1,697	1,422		
Cow 887	Eighth	301	5,778	1,920	1,455	1,967	1,483
	Ninth	297	5,982	2,014	1,511		

Coefficient of variation of directly observed fasting katabolism per square meter of body surface, 4.51±0.51 per cent; per 100 kilograms live weight, 6.65±0.76 per cent.

The experimental routine in the last two experiments mentioned was characterized by one noteworthy innovation—the introduction of the idea of special treatment of the experimental subject to bring about as promptly as possible a condition of true and complete fast.

For this purpose a physic was given to steer No. 260, after which roughage was withheld for one day and grain for one-half day; then the paunch was washed out by means of a stomach pump, and an enema was given. The withholding of roughage for one day seems to have permitted the paunch to clear itself, inasmuch as it appeared to be empty when the pump was used; hence in the preparation of steer No. 259 in experiment 235, and of the three steers (Nos. 254, 36, and 47) in experiment 237, the pumping out of the paunch was omitted. The three steers in experiment 237, therefore, were prepared exactly as was steer No. 259 in experiment 235.

This treatment was more than fairly satisfactory, as indicated by the decrease in feces passed and in methane produced, but inasmuch as further improvement seemed practicable, an effort is being made in experiments now in progress to improve this preliminary treatment by increasing the length of time during which roughage is withheld—the animal being fed grain alone in the meantime.

A further detail of the procedure adopted in the standardization of the treatment preliminary to the determination of the energy metabolism of fast is invariably to have a period of feeding on a maintenance or energy-equilibrium basis immediately preceding the period of fast, thus regularizing the conditions with reference to a possible carrying over into the period of fast of an influence of the preceding plane of nutrition. This procedure was followed in all experiments with both cows and steers recorded in Table 2—that is, the plane of nutrition preceding the fasting period was one of approximate energy balance.

Table 1 shows that the coefficient of variability among 62 computed maintenance requirements was 17.93 ± 1.12 per cent, in terms of square meters of body surface, computed according to Moulton (*22*) and 19.52 ± 1.23 per cent, in terms of kilograms of live weight. These values varied from 662 to 1,643 Calories per square meter of body surface, the largest value being 248 per cent of the smallest.

Obviously such gross inherent variability renders the computation or heat-increment method of determination of the maintenance requirement highly unsatisfactory, and no method of treatment of the data which suppresses such extensive variability can be justified.

Table 2 shows that the coefficient of variability among 18 direct determinations of the maintenance requirement was 4.51 ± 0.51 per cent, in terms of square meters of computed body surface, and 6.65 ± 0.76 per cent, in terms of kilograms of live weight. These values varied from 1,313 to 1,537 Calories per square meter of body surface, the largest value being 117 per cent of the smallest.

The extreme variation of the computed maintenance requirement of an individual animal during the same season is from 836 to 1,411 Calories per square meter of body surface (experiment 216), the largest value being 169 per cent of the smallest, while in the direct determinations such variation is from 1,368 to 1,536 Calories (experiment 237, steer 254) the largest value being 112 per cent of the smallest.

These data show that the directly determined maintenance requirement (fasting heat production) is a fairly satisfactory measure, and further experiments which are in progress are expected to make possible such a standardization of the method of making this estimation as will still further reduce its variability.

CONCLUSIONS

A comparison is made of (1) 62 determinations of the maintenance requirement of energy for cattle, obtained by the heat-increment method, in which the heat production at a status of zero feed intake is computed from the observed heat production and feed intake at two planes of nutrition, and (2) 18 determinations, as the directly observed heat production during fast.

The computed determinations varied between 662 and 1,643 Calories per square meter of body surface, the coefficient of variation being 17.93 ± 1.12 per cent in terms of square meters of body surface and 19.52 ± 1.23 per cent in terms of the live weight.

The direct determinations varied between 1.313 and 1.537 Calories per square meter of body surface, the coefficient of variation being 4.51 ± 0.51 per cent in terms of square meters of body surface and 6.65 ± 0.76 per cent in terms of the live weight.

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The fact that the maintenance requirements as computed from supermaintenance rations are always lower than the directly determined values indicates that the relation between the heat production and the feed intake would not be represented graphically by a straight line and that the utilization of feed energy for maintenance is at a higher rate of efficiency than is its utilization for body increase.

On account of the narrower range of variation of the direct determination it has been adopted as the standard measure of the maintainance requirement of energy in place of the heat-increment method formerly used, and the standardization of this method is in progress.

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THE INHERITANCE OF LENGTH OF STYLE IN BUCKWHEAT[1]

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INTRODUCTION

The flowers of buckwheat (*Fagopyrum esculentum*) are dimorphic. One plant may have flowers with long styles—that is, the styles extend above the stamens, and another may have flowers with short styles. This relationship facilitates cross-pollination by insects, particularly honeybees, which visit the buckwheat flowers in great numbers. The investigation reported here had for its object the determination of the inheritance of length of style.

MATERIAL AND METHODS

Self-fertilization of Japanese and Silverhull buckwheat was induced by covering flower clusters with glassine bags or by covering entire plants with muslin cages. The selfed seed of each individual plant was kept separate and planted in a short row the next year. The seed was spaced along the row so as to permit individual plant study. The length of style of the parent plant and that of the progeny were noted.

In addition to the selfed seed a few seed produced by controlled crossing were obtained. The flowers with similar style lengths on different plants of the same variety were used in making the crosses. A few flowers only were cross-pollinated, but no particular difficulty was experienced in obtaining seed.

EXPERIMENTAL RESULTS

In Table 1 are listed the variety, plant number, year grown, style length of the parent, and number of plants in the progeny with long styles and with short styles, for the material studied during the four years of the experiment. In all, there were examined for style length the progeny of 29 plants that produced one or more selfed seed. The progenies of three plants on which were produced a few seed by artificial crossing were also examined for style length.

Of the self-fertilized plants of Silverhull 10 had short styles, each of which produced progeny that segregated with respect to style length. In all, the progeny consisted of 86 short-styled and 32 long-styled plants. The theoretical ratio on the basis of monohybrid segregation is 88.5 short to 29.5 long. The agreement between the actual and theoretical ratio is close. (Dev./P. E. = 0.8.) Of the

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selfed plants of the Japanese variety 4 had short styles that segregated and produced a total of 19 short-styled and 8 long-styled plants. Here again the agreement between observed and theoretical ratio is close. (Dev./P. E. = 0.8.)

TABLE 1.—*The inheritance of length of style in buckwheat, Fagopyrum esculentum*

Variety	Plant No.	Year grown	Style length of parents	Number of plants in progeny with—	
				Long styles	Short styles
Japanese	1	1923	Long	8	0
Do	2	1923	do	2	1
Do	3	1923	do	13	0
Do	4	1923	Short	1	3
Do	5	1923	do	1	3
Silverhull	6	1923	do	1	0
Do	7	1923	do	1	7
Do	8	1923	do	2	0
Do	9	1923	do	1	7
Do	10	1923	do	1	2
Do	11	1923	do	1	3
Japanese (Long × Long)	12	1923	Long	2	0
Japanese (Short × Short)	13	1923	Short	3	4
Silverhull (Short × Short)	14	1923	do	1	5
Silverhull	15	1924	do	3	10
Do	16	1924	do	8	36
Japanese	17	1925	Long	1	0
Do	18	1925	do	11	1
Do	19	1925	do	0	1
Do	20	1925	do	0	1
Do	21	1925	Short	2	6
Do	22	1925	do	4	7
Silverhull	23	1925	Long	4	0
Do	24	1925	do	7	0
Do	25	1925	do	8	4
Do	26	1925	Short	1	0
Do	27	1925	do	3	4
Do	28	1925	do	7	5
Do	29	1925	do	0	1
Do	30	1925	do	4	8
Do	31	1925	do	0	2
Do	32	1925	do	3	4

Two of the Silverhull short-styled plants (Nos. 29 and 31) produced only short-styled progeny. This fact is of little significance, since the progeny consisted of only three individuals. None of the short-styled plants of Japanese produced exclusively short-styled progeny.

On the assumption that long style is recessive, one would expect the progeny of plants with that character to breed true. In general this expectation was fulfilled, although several exceptions occurred. Perhaps the most outstanding exception was plant 25 of the Silverhull variety, which produced eight long-styled and four short-styled plants. In each of several other cases there was one short-styled individual among the progeny of a long-styled plant The short-styled plants may have resulted from accidental cross-pollination, although considerable precaution was taken to prevent it. It is also possible that genetically long-styled plants may, under certain environmental conditions, become short styled.

The crossed seed produced on plants 12, 13, and 14 gave results in accordance with the assumption that short style is dominant to long style and that a single factor difference controls the inheritance of this character.

The results reported above are in agreement with those obtained by Althausen (1908),[2] Dahlgren (1922),[3] and Egiz (1925).[4]

CONCLUSIONS

The inheritance of style length in *Fagopyrum esculentum* is apparently controlled by a single-factor difference, short styles being dominant to long styles.

[2] ALTHAUSEN, L. ZUR FRAGE ÜBER DIE VERERBUNG DER LANGGRIFFELIGEN UND KURZGRIFFELIGEN BLÜTENFORM BEIM BUCHWEIZEN UND ZUR METHODIK DER VEREDELUNG DIESER PFLANZE. Zhur. Opuitn. Agron. (Russ. Jour. Expt. Landw.) 9: 551–568. 1908. [In Russian, German résumé, p. 568.]

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SELF-FERTILIZATION IN BUCKWHEAT[1]

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INTRODUCTION

The improvement of buckwheat by breeding has not been attempted as extensively as has the improvement of other commonly cultivated field crops, probably because buckwheat is of minor economic importance in the world's agriculture. It is of considerable importance, however, in certain regions owing to the fact that it will yield greater returns on stony, unproductive soil and under cool climatic conditions than will most staple crops.

Two species of buckwheat are cultivated—*Fagopyrum esculentum* and *F. tartaricum*. Most of the buckwheat grown in the United States belongs to the former species. Some investigators name a third species, *F. emarginatum*, but others prefer to consider this form a variety of *F. esculentum*. From the genetic standpoint there is some evidence in support of the latter view.

The appearance of the flowers in the different species of buckwheat is decidedly dissimilar. *Fagopyrum esculentum* and *F. emarginatum* have somewhat showy flowers, which are distinctly dimorphic, whereas *F. tartaricum* has rather insignificant flowers, which are not dimorphic. The flowers of the two species first named are visited much more frequently by insects, particularly honeybees, than are the flowers of *F. tartaricum*. With regard to the number of chromosomes the three species are apparently alike. One of the authors (*6*)[2] has found the diploid number to be 16.

In 1921 an experiment in buckwheat breeding was begun at the West Virginia Agricultural Experiment Station. It was thought necessary first to determine the relative self-fertility of different kinds of buckwheat and to work out a satisfactory method of inducing self-fertilization by artificial means. The purpose of the present paper is to report the results of this phase of the investigation.

MATERIAL AND METHODS

Seed of different varieties of buckwheat was obtained from the United States Department of Agriculture, the University of Minnesota, and farmers in West Virginia. Each lot of seed was planted in a separate row and in a manner such that individual plants could be easily distinguished. The plants which were selfed by bagging flower clusters were spaced at intervals of about 2 inches in rows 1 foot apart, and those which were selfed by covering the entire plant with muslin cages (fig. 1) were spaced at intervals of approximately 30 inches in rows 2 feet apart.

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[2] Reference is made by number (italic) to "Literature cited," p. 190.

Three methods of inducing self-fertilization were tried: (1) Flower clusters were covered with glassine bags just large enough so that each bag covered snugly but one flower cluster; (2) flower clusters were covered with glassine bags large enough to include several flower clusters from the same plant under one bag; (3) entire plants were covered with muslin cages. Some of the covered flowers were manipulated by rubbing between the thumb and forefinger without removing the glassine bags, and others were actually self-pollinated by rubbing the ripened anthers of a certain flower on the stigmatic surface of the same flower.

The glassine bags used for covering the flower clusters were made of high-grade paper, the edges of which were fastened with waterproof glue. The cages were constructed by building frames of suitable

FIG. 1.—Muslin cages used to cover individual buckwheat plants

dimensions and covering them with good-quality muslin. Some idea of their construction may be gained by examining Figure 1. After each muslin cage was placed in position over a single plant, a small ridge of soil was thrown up around the lower edge of the cage to exclude insects.

RELATIVE SELF-FERTILITY OF FAGOPYRUM ESCULENTUM AND F. TARTARICUM

The work of Althausen (*1*, *2*, *3*), Stevens (*7*), Dahlgren (*4*), Egiz (*5*), and others has proved beyond doubt that it is possible to self-fertilize *Fagopyrum esculentum*. The investigator first named pointed out that *Fagopyrum tartaricum* was spontaneously self-fertile. Similar results have been obtained at the West Virginia Agricultural Experiment Station.

In Table 1 are shown the different varieties, the year grown, the number of plants selfed, the number of plants which set seed, the total number of seeds set, and the range in number of seeds set per plant. The plants were selfed in various ways, but most of them were selfed by covering one or more flower clusters with a large glassine bag. The results, however, are comparable, as all the strains were treated approximately the same, except that in only a few instances were cages placed over plants belonging to the *tartaricum* species.

It is at once apparent from Table 1 that varieties of *tartaricum* set seed in abundance. In fact no appreciable difference in quantity of seed set was noted between flower clusters which were covered and those which were not. Sixteen plants of Mountain (Cereal Investigation No. 91) set a total of 817 selfed seed in 1921, and in most cases only one or two flower clusters were covered on a plant. The next year 8 of 10 plants of this variety set a total of 256 selfed seed. Two strains of rye buckwheat and a sort grown under the name of Tartarian also set considerable selfed seed in 1922. These varieties all belong to *Fagopyrum tartaricum*.

TABLE 1.—*Number of selfed seeds set by different varieties of two species of buckwheat*

Variety	Species	Year grown	Number of plants selfed	Number of plants setting seed	Total number of seeds set	Number of seeds set per plant
Mountain (C. I. 91)	Fagopyrum tartaricum	1921	16	16	817	10 to 119
Japanese	Fagopyrum esculentum	1921	27	2	6	1 to 5
Silverhull	do	1921	14	1	1	1
Mountain (C. I. 91)	Fagopyrum tartaricum	1922	10	8	256	----------
Rye (C. I. 79)	do	1922	6	5	211+	----------
Rye (Minnesota)	do	1922	8	7	197	----------
Tartarian (Minnesota)	do	1922	11	10	108	----------
Japanese	Fagopyrum esculentum	1922	52	11	61	1 to 21
Silverhull	do	1922	60	10	45	1 to 20
Gray (Minnesota)	do	1922	16	3	40	1 to 32

The results of self-fertilizing Japanese, Silverhull, and Gray buckwheat, all varieties of *Fagopyrum esculentum*, are also shown in Table 1. In 1921, of 27 selfed Japanese plants, 2 set a total of 6 seeds, and of 14 selfed Silverhull plants only 1 set 1 seed. In 1922 there were selfed 52 Japanese, 60 Silverhull, and 16 Gray plants, of which 11 Japanese, 10 Silverhull, and 3 Gray produced a total of 61, 45, and 40 seeds, respectively. A few of these selfed plants produced more than 20 seeds each, as may be seen from the last column of Table 1. It is obvious from the above-mentioned facts that although varieties of *F. esculentum* do not set selfed seed as abundantly as varieties of *F. tartaricum*, nevertheless, they will set some seed under conditions which induce self-fertilization.

METHODS OF SELFING

In 1922 the relative efficiency of large and small glassine bags over flower clusters and of muslin cages over entire plants in bringing about self-fertilization was determined. The data are presented in Table 2.

TABLE 2.—*Number of seeds of buckwheat (Fagopyrum esculentum) produced by different methods of selfing in 1922*

Variety	Flower clusters under—						Plants under muslin		
	Large glassine bags			Small glassine bags					
	Number of plants	Number of plants setting seed	Total number of seeds	Number of plants	Number of plants setting seed	Total number of seeds	Number of plants	Number of plants setting seed	Total number of seeds set
Japanese	27	6	16	16	0	0	9	5	45
Silverhull	31	3	15	20	0	0	9	7	38
Gray	10	1	1	4	0	0	2	2	39

Flower clusters on 27 Japanese, 31 Silverhull, and 10 Gray plants were covered with large glassine bags. Of these, 6 Japanese, 3 Silverhull, and 1 Gray set a total of 16 seeds, 15 seeds, and 1 seed, respectively. Under similar conditions flower clusters on 16 Japanese, 20 Silverhull, and 4 Gray plants were covered with small glassine bags, but not a single seed was obtained. The results with muslin cages were more satisfactory. Fourteen of the 20 plants covered set a total of 122 seeds. When one considers the fact that the muslin cages covered entire plants and the large glassine bags covered only a few flower clusters on each plant, the difference, if any, in the efficiency of the two methods in producing self-fertilization is not very striking. Moreover, the use of muslin cages is rather expensive and only relatively few plants may be covered.

Some of the flowers which had previously been covered were manipulated by being rubbed gently between the thumb and forefinger. No striking increase in the number of seeds set resulted from this treatment. Other flowers were selfed by rubbing ripened anthers on the stigmas by means of a tweezer or small swab. The swab was made by wrapping a bit of cotton around the end of a toothpick. Some seeds were set as a result of this treatment but not enough to justify its use for producing selfed seed in quantity.

RELATIVE SELF-FERTILITY OF LONG-STYLED AND SHORT-STYLED PLANTS

In order to determine whether length of style was correlated with the number of selfed seeds set in varieties of *Fagopyrum esculentum* the data presented in Table 3 were collected during 1922, 1923, 1924, and 1925. The variety, the year grown, the total number of plants selfed, the number that set seed, the total number of seeds, and the range in number of seeds set per plant by long-styled and short-styled plants are shown.

During the four years included in the experiment three varieties were under observation. Of 124 long-styled plants which were selfed, 22 set a total of 107 seeds, and of 116 short-styled plants, 40 set a total of 281 seeds. If only the plants that set seeds are counted, the short styled plants average approximately 7 seeds per plant and the long-styled plants 5 seeds per plant. Moreover, the relative number of plants that set selfed seed among the short-styled plants

was approximately double that among the long-styled plants. In this experiment it was apparently somewhat easier to obtain selfed seed from short-styled plants than from long-styled plants. In flowers of the former the stigmatic surface is beneath the anthers, whereas in flowers of the latter the positions are reversed. The chances that pollen will reach the stigmas of covered flowers on short-styled plants are somewhat greater than that it will reach covered flowers of long-styled plants.

TABLE 3.—*Number of selfed seeds produced by buckwheat plants (Fagopyrum esculentum) with long and with short styles*

Variety	Year grown	Long styles				Short styles				Method of selfing
		Total number of plants selfed	Number of plants setting seed	Total number of seeds	Number of seeds set per plant	Total number of plants selfed	Number of plants setting seed	Total number of seeds	Number of seeds set per plant	
Japanese	1922	16	3	28	2 to 21	20	7	25	1 to 12	Some plants covered entirely with a muslin cage; on others only a flower cluster covered with a glassine bag.
Silverhull	1922	28	5	8	1 to 3	20	5	37	1 to 20	
Gray	1922	11	1	7	7	3	2	33	1 to 32	
Japanese	1923	4	1	1	1	5	3	5	1 to 2	Each plant covered with a muslin cage.
Silverhull	1923	5	0	0	0	6	2	64	13 to 51	
Gray	1923					1	1	3	3	
Japanese	1924	13	2	2	1	13	1	2	2	Flower clusters only covered with glassine bags.
Silverhull	1924	16	1	1	1	21	6	60	1 to 29	
Japanese	1925	12	4	20	1 to 16	10	4	16	1 to 10	Do.
Silverhull	1925	19	5	40	2 to 19	17	9	36	1 to 12	Do.
Total		124	22	107		116	40	281		

RELATIVE SELF-FERTILITY OF JAPANESE, SILVERHULL, AND GRAY BUCKWHEAT

Table 3 shows the relative self-fertility of the different varieties of *Fagopyrum esculentum*. In all there were selfed 93 plants of Japanese, 132 of Silverhull, and 15 of Gray buckwheat. Of these, 25 Japanese, 33 Silverhull, and 4 Gray plants set a total of 99, 246, and 43 seeds, respectively. The 4 plants of Gray which set seed averaged about 11 seeds per plant. The number of plants worked with in this variety was not sufficient for a random sample. Of the plants which set selfed seed in the other two varieties, Japanese averaged about 4 seeds per plant and Silverhull about 7 seeds per plant. These data indicate that the flowers of Silverhull when covered set somewhat more seed than do the flowers of Japanese when covered.

SUMMARY AND CONCLUSIONS

Several varieties of *Fagopyrum esculentum* and *F. tartaricum* were self-fertilized. Varieties of the latter proved much more highly self-fertile than varieties of the former.

Different methods were used in covering the flowers and in inducing self-fertilization. Large glassine bags and muslin cages were

found to be most satisfactory. The manipulation in various ways of previously covered flower clusters did not result in a marked increase in the amount of selfed seeds set.

The relative self-fertility of short-styled and long-styled plants of *Fagopyrum esculentum* as measured by the number of selfed seeds set was determined. Short-styled plants proved to be somewhat more self-fertile than long-styled plants.

In the experiment described in this paper relatively more selfed seeds were produced by Silverhull buckwheat than by Japanese.

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EFFECT OF SMUT (USTILAGO ZEAE) ON THE SUGAR CONTENT OF CORNSTALKS[1]

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INTRODUCTION

It has recently been shown (*3*) [2] that the specific gravity of the juice from cornstalks infected with *Ustilago zeae* is lower than that of the juice of comparable healthy stalks. The further observation that the juice from sound stalks is sweeter than that from diseased stalks suggested that the lower specific gravity of the juice from the diseased stalks is brought about by the removal of sugar by the smut fungus. This suggestion seemed all the more probable since it appears from the analyses given by Collier (*1*) that differences in the specific gravity of the juice of cornstalks, and of sorghum as well, are mainly attributable to differences in the sugar content of the juice. From these considerations it appeared likely that a comparative examination of the sugar content of sound and of diseased stalks might yield some information on the mode of nutrition of the corn-smut fungus.

MATERIALS AND METHODS

Field corn for this purpose was grown on the Arlington Experiment Farm, through the courtesy of C. H. Kyle, agronomist in Corn Investigations. Material for the analyses was collected between September 11 and September 18, 1925. During that week the plants matured rapidly, so that the kernels were in the "hard-dough" stage at the final cutting. For diseased material, stalks were selected which had one or more smut galls of different sizes at the nodes. Inasmuch as corn plants of a single variety growing in the same field may differ greatly in sugar content, a healthy control plant similar in size, vigor, number of internodes, and state of maturity was selected with each diseased plant. The control plants were taken from hills adjacent to the corresponding diseased plants.

The selected stalks constituting each pair were taken to the laboratory together, and handled as quickly as possible. From the diseased stalk an internode adjoining a smut gall, or in the case of a heavily smutted stalk, an internode between two galls was selected, wiped clean with a damp cloth, and cut into slices about 1 mm. thick with a rotary slicer. Fifty grams of the material were weighed out and preserved in 95 per cent alcohol, with the addition of 0.5 gm. of calcium carbonate. The corresponding internode of the control plant was then similarly sampled.

For the determination of dry matter and sugars the individual samples were handled in the following manner. The alcohol in which the sample had been preserved was poured off through a

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[2] Reference is made by number (italic) to "Literature cited," p. 194.

weighed filter into a 1,000-ml. flask. The solid residue was transferred to a beaker and extracted five times by boiling for 10-minute periods in 70 per cent alcohol. After each boiling the extract was decanted through the filter into the flask. After the last decantation, the weighed filter with the solid residue was transferred to a tared aluminum weighing can. The weight of the dry residue was obtained by evaporating the free alcohol at 65° C. and drying the residue to constant weight at 105°. For the determination of solids in the alcoholic extract an aliquot was evaporated at a low temperature in a can containing sand, and dried to constant weight in a vacuum oven at 70°. Reducing sugar and total sugar were determined in another aliquot of the alcoholic extract which had been freed from alcohol by evaporation, cleared with neutral lead acetate, and freed from lead by means of sodium oxalate. The reductions were carried out with strict observance of the details of manipulation described by Munson and Walker (*6*). The cuprous oxide was weighed as such.

TABLE 1.—*Sugar content of smutted and of healthy cornstalks*

Pair No.	Date of cutting	Internode (numbered from base of stalk)	Percentage of dry matter		Reducing sugar as glucose		Sucrose		Total sugar as glucose	
			Healthy plant	Smutted plant	Healthy plant	Smutted plant	Healthy plant	Smutted plant	Healthy plant	Smutted plant
			Heavy infection							
	1925				*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Sept. 11	7	20.66	17.48	13.7	11.9	22.1	13.0	37.0	25.6
		3	17.78	14.17	17.6	15.7	15.7	5.7	34.1	21.7
2	Sept. 14	5	20.76	17.13	15.9	12.6	14.8	4.3	31.5	17.1
3	Sept. 11	8	22.16	18.01	14.3	11.4	19.5	10.5	34.8	22.4
4	do	3	19.00	16.23	16.2	15.5	17.6	5.6	34.7	21.4
5	Sept. 18	8	20.37	21.21	9.3	6.7	26.7	10.0	37.4	17.2
		3	17.11	14.54	11.5	2.1	22.8	3.7	35.5	6.0
			Medium infection							
6	Sept. 10	5	19.16	16.69	12.4	10.3	10.7	8.7	23.7	19.5
7	Sept. 14	7	17.91	17.35	15.1	12.8	5.5	2.4	20.9	15.3
8	do	8	17.47	17.77	16.0	14.1	7.8	10.8	24.2	25.5
9	Sept. 17	5	17.51	19.51	9.5	8.3	7.6	6.6	17.5	15.2
10	do	5	18.78	16.92	17.5	9.9	7.6	1.9	25.5	11.8
11	Sept. 14	7	15.68	16.31	18.2	12.0	6.1	9.0	24.6	21.5
			Slight infection							
12	Sept. 10	4	24.01	26.04	12.5	12.7	24.0	19.8	37.8	33.5
13	Sept. 17	5	22.79	22.78	14.1	12.1	13.8	9.9	28.6	22.5
14	Sept. 14	7	19.21	17.49	15.8	16.7	13.9	4.8	30.4	21.8

RESULTS

The results of the determinations are given in Table 1, in which the sugar content of the stalks is expressed in percentage of dry matter. The data are presented in three groups, according to the degree of infection of the diseased stalks. The diseased plants of the first group, under "heavy infection" each had three or more large galls

on the stalks. Those of the third group, under "slight infection," had one or two small galls only. Those of the second group, under "medium infection," had one or two fairly large galls and usually one or two small ones, so that in the writers' estimation they represent a degree of infection intermediate between that of the other two groups.

It appears from these data that the growth of *Ustilago zeae* on the stalks of corn results in a considerable reduction of sucrose in the diseased stalks, and an evident, though lesser, reduction of hexoses, the loss of sucrose being greatest in the most severely infected stalks. There are several exceptions among the less heavily smutted plants. Owing to the variability of the sugar content of normal plants, discrepancies of this kind may be expected in view of the difficulty of pairing plants by their appearance. This variability is sufficient to render detection of the effect of slight infections uncertain.

DISCUSSION

While the mode of nutrition of saprophytic fungi and of facultative parasites has been extensively investigated, our knowledge of the nutrition of obligate parasites is still comparatively meager. Incidental observations which may be regarded as having a bearing on the question are found in the literature, although they are not always interpreted from this point of view. Thus, the accumulation of starch in association with rust pustules and in tissues hypertrophied as a result of rust infection has frequently been noted.

Perhaps the earliest observation indicating definitely the utilization of starch by an obligate parasite is that of Reess (*10*), who states that the starch accumulated in the leaves of *Pinus picea* [*Picea excelsa*] as a result of infection by *Chrysomyxa abietis* is consumed by the fungus at the time of spore formation. The disappearance of starch from tissues infected by different rust fungi was noted also by Peglion (*7*), Robinson (*11*), and Mains (*4*).

The observation, frequently made, that rusts develop less vigorously on shaded plants than on plants growing in full light has been considered as showing a nutritive relation between the products of photosynthesis of the host and the growth of the parasite. Direct experimental evidence of the dependence of rusts upon the carbohydrates of their hosts or upon intermediate products of photosynthesis was first furnished by the work of Ward (*13*), who showed that the hyphae of *Puccinia glumarum* soon starve and degenerate in leaves of wheat maintained in an atmosphere free from carbon dioxide. Later Fromme (*2*) found that *P. coronifera* does not develop on its host in the absence of light. The most conclusive evidence of this relation is furnished by the extensive experiments of Mains (*4*), who showed that in the absence of light or of carbon dioxide the development of *P. coronata* and *P. sorghi* on their hosts is retarded or stopped on account of lack of carbohydrates. If, however, carbohydrates be supplied by means of sugar solutions, *P. sorghi* develops in the dark on seedlings of maize deprived of their endosperms and also on pieces of corn leaves floating on the solutions. Other observations showing the dependence of rusts on the carbohydrate supply of the host have been reported by

Waters (*14*) for *Uromyces appendiculatus* and by Peltier (*8, p. 51; 9, p. 8–11*) for *P. graminis tritici*.

Also of interest is the work of Tischler (*12*) who, in his extended study of the influence of *Uromyces pisi* on *Euphorbia cyparissias*, found that although the hyphae of the fungus penetrate the meristematic tissue of the growing point which contains no sugar, haustoria are formed only in the older vacuolate cells which are rich in starch and sugar. While Tischler interprets this observation in the sense of Miyoshi's (*5*) experiments, it seems that the formation of haustoria in the tissues rich in sugar may be regarded as an indication of the nutritive relations of the fungus.

The writers' data show that the growth of *Ustilago zeae* on cornstalks results in a large reduction of the sugar content of the tissues. It seems evident, therefore, that this fungus also depends on the carbohydrates of the host for a part of its nutriment.

The data do not permit any definite conclusions as to the form in which the carbohydrates are absorbed by the fungus, although they suggest some possibilities. The great difference in sucrose between the healthy plants and the smutted plants might be interpreted as indicating that the fungus uses this sugar primarily. However, the low sucrose content of the infected plants might come about through utilization of the hexoses only. In that case the low sucrose content of diseased plants may be explained on the assumption that the formation of sucrose is hindered by the withdrawal of hexoses, or that sucrose undergoes inversion when the removal of the hexoses disturbs the equilibrium between sucrose and hexoses in the cells.

In the case of rusts it has been suggested that the fungus absorbs intermediate products which occur in the process of carbohydrate formation. This possibility would seem to be excluded in the case of *Ustilago zeae*, since the fungus can invade the young kernels, in which, so far as we know, primary synthesis of carbohydrates does not take place, but to which the carbohydrates are transported as such.

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